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R. Guy Riefler, Sushma Guruswamy, and Victor F. Medina,

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Department of Civil Engineering
Ohio University
141 Stocker Center
Athens, Ohio 45701

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UPTAKE AND TRANSFORMATION OF THE PROPELLANTS 2,4-DNT, PERCHLORATE AND NITROGLYCERIN BY GRASSES

by

Guy Riefler[1], Sushma Guruswamy[1], and Victor Medina[2]

[1]Department of Civil Engineering
Ohio University
Athens, Ohio 45701

[2]U.S. Army Corps of Engineers
Engineer Research & Development Center
Vicksburg, Mississippi 39180

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Abstract

UPTAKE AND TRANSFORMATION OF THE PROPELLANTS 2,4-DNT, PERCHLORATE AND NITROGLYCERIN IN GRASSES (98 pp.)

The feasibility of using grasses, yellow nutsedge (*Cyperus escalantus*), Indian grass (*Sorghastrum nutans*) and big bluestem (*Andropogon gerardii*) in the clean-up of propellant contaminated soil was assessed by performing hydroponic and soil experiments. The propellants 2,4-dinitrotoluene (2,4-DNT), perchlorate and nitroglycerin (GTN) often contaminate firing ranges due to partially detonated ordnances and incomplete combustion of munitions.

In hydroponic experiments, sorption of 2,4-DNT to roots was significant in the grasses. Perchlorate accumulated in large amounts in leaves of the grasses with maximum accumulation in yellow nutsedge (732 mg kg^{-1}). GTN was removed from the solution rapidly and almost 100% degradation was seen in all grasses within 52 hours. From soil experiments, it is evident that yellow nutsedge was effective in degrading 2,4-DNT. Perchlorate was accumulated in leaves of yellow nutsedge and Indian grass and GTN degradation was nearly 100% in Indian grass. Degradation in perchlorate and GTN was by bacteria.

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1. Introduction

Since 1802, the US army has seen a sizable increase in the number of candidates joining the military services which has resulted in a larger area of land being used for practice and training. Soldiers often practice with live munitions in order to improve their performance during combat, which sometimes leaves live munitions either partially detonated or in a state of incomplete combustion. This has led to the accumulation of explosives in soil causing the contamination of soil at firing ranges. Leaching of the surface explosives sometimes results in contamination of ground water. Another source of contamination of soil and groundwater by explosives is improper disposal of waste from the explosive industry, which until the 1980's was often discarded in unlined pits (Rodgers et al., 2001). The issue of surface contamination near firing ranges and explosive industries was not addressed seriously until recently.

The most commonly found propellants in contaminated soil near firing ranges are 2,4-dinitrotoluene (2,4-DNT), perchlorate, and nitroglycerin (glycerol trinitrate - GTN). Propellants are explosives used in firing projectiles from weapons. 2,4-DNT and GTN are commonly found in bullets and cannon ordnance while perchlorate is the primary oxidant in rocket propelled grenades, larger missiles and rockets (Pennington et al., 2003).

Because the US army trains continuously, clean-up of contamination is complicated by the fact that closing down the site for remediation is not possible. In addition, the presence of partially detonated ordnances makes the clean-up process more challenging. Phytoremediation is potentially an attractive remedial measure for clean-up of propellant contaminated soil because it can be implemented without disturbing base

operations and will minimize the risk of unexploded ordnance detonation during implementation.

1.1. Phytoremediation

Over the last 15 years, the use of plants in removing xenobiotics and heavy metals from sludges, sewage waters, spillage sites and polluted areas has become an important area of research. Extensive work is being done to study the transformation of these contaminants by plants (Harvey et al., 2002).

Phytoremediation is a technique to stabilize and remediate contaminants in soil by using plants. There are five mechanisms involved in phytoremediation, including phytoaccumulation, phytodegradation, phytovolitilization, phytostabilization and rhizodegradation. Phytoaccumulation involves the accumulation of contaminants in the harvestable part of plants in order to remove it from the soil. The harvestable parts may include seeds, fruits or roots. In phytodegradation, plants, along with microorganisms, degrade the contaminant present in the soil. Degradation in the plant is carried out by enzymes present in the plant tissue or by bacteria that inhabit the plant. Phytovolitilization is the use of plants to volatilize the contaminants present in the soil. Volatilization of contaminants may also occur during transpiration of water by plants. Phytostabilization reduces the bioavailability of a contaminant, thus preventing its entry into the groundwater or food chain, by adsorbing or precipitating it into the soil (Nzengung et al., 1999).

Biodegradation of contaminants may occur in the root zone or rhizosphere of some plants due to the presence of plant enzymes, plant exudates, and bacteria. A thriving bacterial community often forms in the rhizosphere with populations and

activities over ten times greater than surroundings (Nzengung et al., 1999). This relationship is often symbiotic with the plant releasing exudates to provide growth substrate to the bacteria and the bacteria providing nutrients to the plant. Biodegradation by these processes is called rhizodegradation (Nzengung et al., 1999).

Phytoremediation is more widely accepted than other remediation technologies in treating explosive contaminated sites as it is less expensive, can cover large areas of land and is non-invasive (Rosser et al., 2000). It does not involve any environmental disturbance but requires more time for plants to grow. Plants are chosen for cleaning up contaminants according to their seasonal availability, root depth and penetration through the soil.

Currently, phytoremediation is being applied to hazardous waste sites where other treatment methods are too expensive or impractical. It is also being applied to low level contaminated sites which require "polishing treatment" over a longer period of time (Dietz and Schnoor, 2001). Phytoremediation has also been studied at sites contaminated with chlorinated solvents, pesticides and metal pollutants. Use of poplar and willow trees in phytoremediation has been effective in clean-up of metals, organic pollutants and waste sites. Recently, transgenic plants have been engineered to detoxify and transform xenobiotics compounds (Dietz and Schnoor, 2001).

Grasses are an ideal choice for phytoremediation of propellant contaminated sites because they stabilize and prevent contamination from spreading. Grasses native to a region tend to grow rapidly and can be easily cultivated without digging at the sites. Yellow nutsedge (*Cyperus escalantus*), Indian grass (*Sorghastrum nutans*) and big bluestem (*Andropogon gerardii*) were selected for this study as they are commonly found

throughout the United States and they represent a range of habitats. These are native wild species which compete within their native habitats and are considered non-invasive.

1.2. 2,4-DNT

2,4-DNT is a nitroaromatic explosive with two nitro groups on a benzene ring (Figure 1). It has a tendency to partition in the soil (log K_{ow} = 1.98) and is soluble at a concentration as high as 270 mg/L. The presence of two electron-withdrawing nitro groups makes 2,4-DNT resistant to chemical or biological oxidation and to hydrolysis (Rodgers et al., 2001).

Apart from being used in the production of explosives, 2,4-DNT is used as an intermediate in the manufacture of polyurethanes, dye processes and in smokeless gunpowders (U.S.EPA, 2006). Even though 2,4-DNT has not been classified as a potential carcinogen by the United States Environmental Protection Agency (U.S.EPA), studies show that it could cause acute effects in humans, and cyanosis and ataxia in animals (U.S.EPA, 2006). Long term exposure to 2,4-DNT may cause damage to the central nervous system.

Bacterial transformation of 2,4-DNT into 2,4-diaminotoluene (2,4-DAT) takes place under anaerobic conditions (Cheng et al., 1996). The nitro group reduces to 2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene with highly reactive nitroso and hydroxylamino groups as intermediate compounds (Figure 1). Complete reduction of both of these nitro groups to amino groups takes place under anaerobic conditions (McCormick et al., 1978). But, this degradation product is unstable, as the 2,4-DAT undergoes spontaneous complete degradation under aerobic conditions (Cheng et al., 1996).

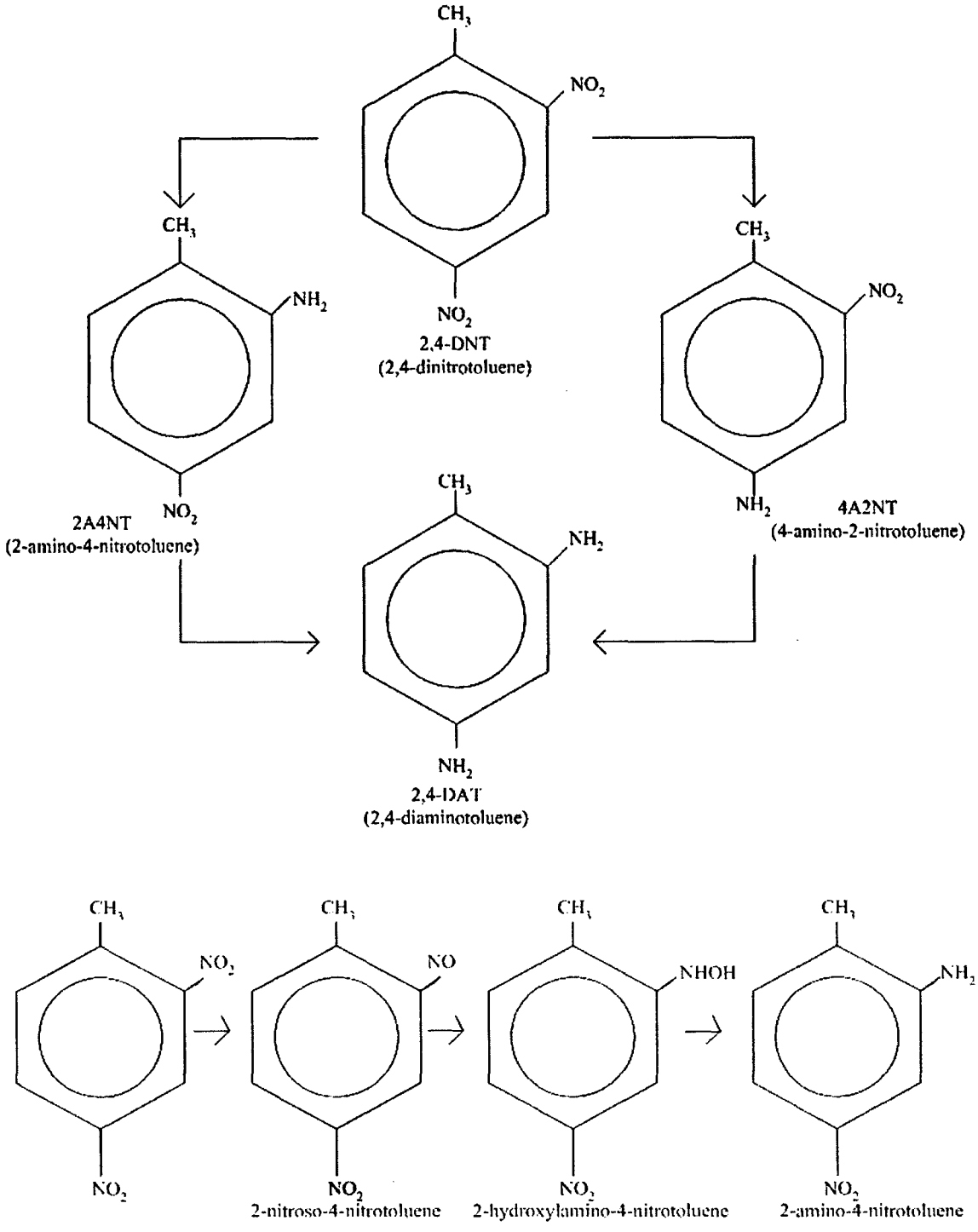

Figure 1: 2,4-DNT degradation pathway

Reduction of the 4-nitro group is favored under low 2,4-DNT concentration while the 2-nitro group is reduced first under high 2,4-DNT concentration (Cheng et al., 1996). Biotransformation of 2,4-DNT does not take place at very high concentrations (Cheng et al.,1996). Bacterial transformation of 2,4-DNT has been observed under both aerobic and anaerobic conditions (Nishino et al., 2000; Hughes et al., 1999). Under aerobic conditions, certain microbial strains accumulate hydroxylamino intermediates which eventually undergo oxidative coupling to form azoxy compounds ((McCormick et al., 1978; Nishino et al., 2000).

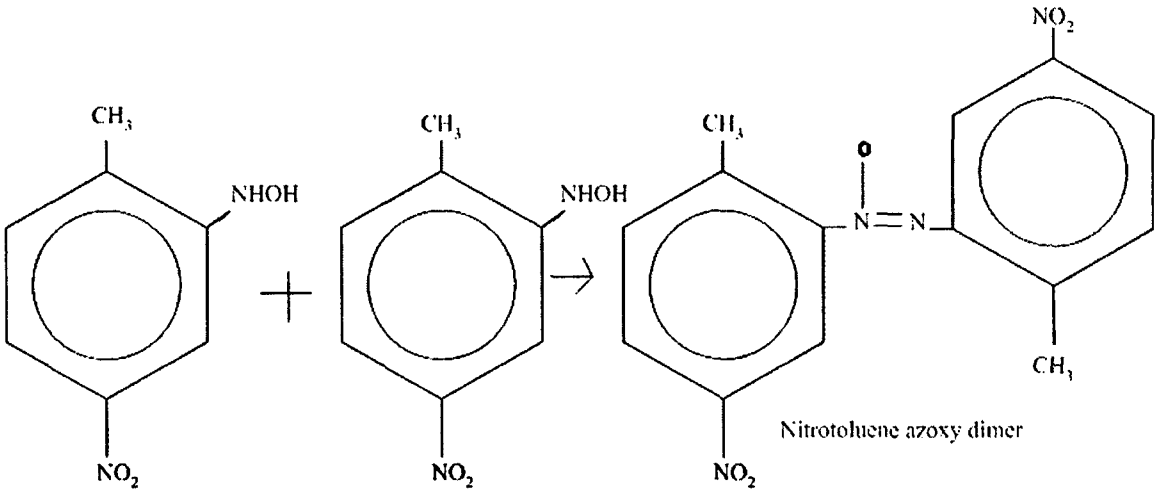

Figure 2: Oxidative coupling of hydroxylamino intermediates to for azoxy compounds

2,4-DNT can also be transformed by plant based enzymes (Rodgers and Bunce, 2001). Nitroreductases and laccases aid in phytodegradation of nitroaromatics in plants (Rodgers and Bunce, 2001). Data available on the uptake of 2,4-DNT in plants is limited, but its degradation rate is assumed to be comparable to that of higher explosives like TNT (Brannon and Pennington, 2002).

1.3. Perchlorate

Perchlorate (ClO_4^-) is formed by dissolving perchloric salts with cations like ammonium, potassium, and sodium (Motzer, 2001). It is kinetically stable and very mobile (log K_{ow} of ammonium perchlorate = -5.84). Reducing a chlorine atom from the + 7 oxidation state to the - 1 oxidation state requires energy in the form of heat or light, or a catalyst to aid the reaction. Hence, even in highly reducing subsurface environments, chemical reduction of perchlorate chlorine is not observed (Motzer, 2001). Due to its high mobility, perchlorate salts have a tendency to migrate down aquifers and settle in confining layers. Degradation of perchlorate in these layers may take more than 100 years by either mass transfer or dilution (Motzer, 2001). Studies show that in the U.S. more perchlorate is present in soil and groundwater than surface water (Nzengung et al., 2004). Perchlorate degrades to chloride with chlorate and chlorite as intermediates (Figure 3).

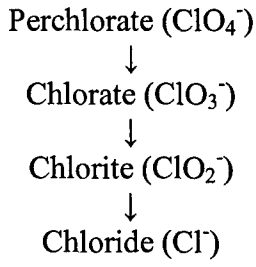

Figure 3: Anaerobic degradation pathway for perchlorate

Phytodegradation of perchlorate is carried out by plant associated microbes in the rhizosphere and also by the presence of enzymes like perchlorate reductases and chlorite dismutases in plant tissues (Van Aken and Schnoor, 2002). The rhizosphere tends to be aerobic but may contain anaerobic zones (Nzengung et al., 2004). Aerobic conditions tend to slow down transformation of perchlorate by reductive enzymes when compared to degradation of perchlorate by anaerobic bacteria (Van Aken and Schnoor, 2002).

Sorption of perchlorate to soil and sediments depend on pH and ionic strength. No sorption occurs with sandy soil under most conditions, and at near neutral pH sorption is considered insignificant (Motzer, 2001).

Perchlorate is most commonly used in rocket and missile engine propellants including hand held rocket propelled grenades (RPGs), in the form of ammonium perchlorate salt, as an oxidant. Because perchlorate is less sensitive to shock and environmental changes they are used extensively by the explosive and propellant industries (Nzengung et al., 1999). It is also used as an oxidizer, in power generation, as a drying agent for gases, in electrolytic baths, jet fuels, and as a chemical intermediate.

Perchlorate has detrimental effects on the human body. Since its ionic radius and charge is similar to that of iodine, there is a significant reduction in thyroid iodine uptake in the body (Dasgupta et al., 2005). Perchlorate causes irritation in skin, eyes and mucous membranes. In the form of dust and vapors, it causes coughing and shortness of breath. Even though there is no maximum contaminant level (MCL) set for perchlorate, it has been included in the Drinking Water Contaminant Candidate List or CCL (U.S.EPA, 1998). Detection of perchlorate in drinking water in Arizona, California, Nevada and Texas has forced these states to set perchlorate drinking water standards given in Table 1. The U.S.EPA has a perchlorate regulatory level of 1 μg/L in drinking water which is under scrutiny as other U.S. federal agencies have found this to be baseless and unrealistic (Dasgupta et al., 2005). A National Academy of Science (NRC) committee has set drinking water standards for perchlorate to be 24.5 μg/L with an oral reference dose of 0.7 $\mu g\ kg^{-1}\ d^{-1}$.

Table 1: Current state regulatory agency perchlorate in drinking water action levels (Motzer, 2001)

State	Regulatory agency	Type of action	Implementation date	Perchlorate action level in µg/L
Arizona	Arizona Department of Environmental Quality (ADEQ)	Health Based Guidance Level	May 2000	14
California	California Department of Health Services (DHS)	Health Based Action Level (based on toxicity)	1997	18
Nevada	Nevada Division of Environmental Protection (DEP)	Health Based Action Level	August 1997	18
Texas	Texas Natural Resource Conservation Commission (TNRCC)	Interim Action Level	January 1999	22

Phytoremediation of perchlorate has been studied extensively. Transformation of perchlorate was evident in the rhizosphere of plants due to the presence of bacteria which thrive on root exudates and initiate perchlorate degradation (Shrout et al., 2006). Perchlorate was also taken-up into leaves (phytoaccumulation) of willow trees, and in the roots perchlorate was phytodegraded to form chloride, with chlorate and chlorite as intermediate degradation products (Nzengung et al., 2004). Aerobic and high nitrate conditions were favorable for increased uptake of perchlorate in plants (Nzengung et al., 2004). Large proportions of perchlorate were found not only in leaves but also in other parts of sweet gum and willow trees suggesting the possibility of translocation of metabolites upon their production in leaves or transformation of perchlorate by other plant cells (Van Aken and Schnoor, 2002). Uptake of perchlorate has also been seen in poplar trees (Van Aken and Schnoor, 2002). In smartweed, perchlorate uptake was by plant transpiration (Tan et al., 2006). Uptake of perchlorate was analyzed in terrestrial plants like cucumber (*Cucunis sativus* L.), lettuce (*Lactuca sativa* L.) and soybean (*Glycine max*) (Yu et al., 2004). Although perchlorate accumulated in the leaves, the uptake of perchlorate differed from one plant to another. Lettuce was found to accumulate larger amounts of perchlorate when compared to cucumber and soybean (Yu

et al., 2004).The rate of uptake was dependent on the level of nutrients in the soil. Woody plants like eastern cottonwoods (*Populus deltoides*), *Eucalyptus cineria* and willow (*Salix nigra*) were analyzed for uptake and transformation of perchlorate (Nzengung et al., 1999). From the study, *E.cineria* was found to have the fastest kinetics with 46 % of perchlorate found in leaves and 6.6 % in roots in 26 days (Nzengung et al., 1999). Phytoremediation of perchlorate depended upon the concentrations of competing terminal electron acceptors (TEAs) like NO_3^- (Nzengung et al., 1999).

1.4. Nitroglycerin (GTN)

Like 2,4-DNT, GTN has nitro functional groups (Figure 4). GTN is persistent and mobile at contaminated sites like other propellants. It has a tendency to partition into soil with a log Kow value of 1.62. It is highly toxic and is used as an energy plasticizer for both gun and rocket propellants (Bhaumik et al., 1996). Apart from being used in military applications, it is also used in the field of medicine for the treatment of blood pressure and heart diseases (Brodman et al., 1997).

The biodegradation pathway for GTN by bacteria is given in Figure 4.

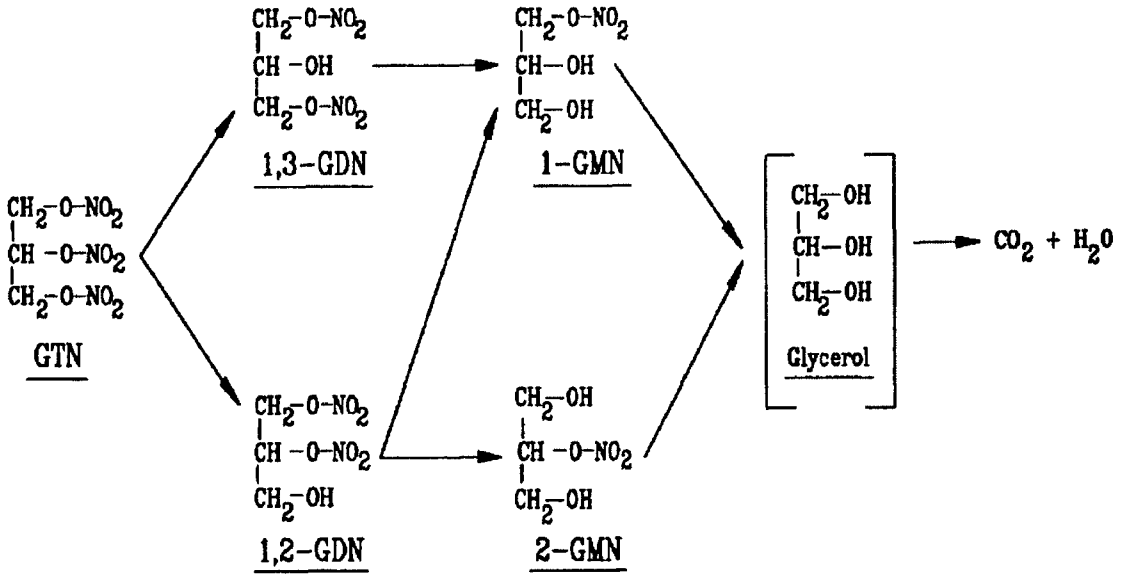

Figure 4: Biodegradation pathway of GTN (Christodoulatos et al., 1997)

Both aerobically and anaerobically, GTN undergoes biochemical degradation by successive denitrations resulting in complete mineralization. In anaerobic biodegradation, the amounts of byproducts formed during denitration, namely nitrate and nitrite, are reduced to nitrogen gas by a large extent (Christodoulatos et al., 1997). Aerobic degradation of GTN does not result in complete mineralization. Some amount of glycerol dinitrates (GDN) and glycerol mononitrates (GMN) accumulate which suggests that aerobic degradation proceeds at slower rates (Accashian et al., 1998). Since GDN and GMN are mutagenic, complete removal of both is required in order to successfully detoxify GTN (Bhaumik et al., 1997). In the presence of microbial cell cultures and suitable substrates, complete mineralization of GTN is possible.

GDN is a powerful explosive like GTN with its shock sensitivity barely less than GTN (Bhaumik et al., 1996). GMN is a weak explosive and has extremely low impact sensitivity.

Plants have been studied in the past to analyze the interaction between plant cells and their ability to degrade GTN (Goel et al., 1996). Degradation of GTN was observed within 35 h from the start of experiment suggesting a possibility of complete degradation by plants (Goel et al., 1996). Microbial degradation in the rhizosphere of plants took place by constant secretion of exudates and plant enzymes by roots. Plant enzymes and exudates provided the necessary substrate for the degrading bacteria to thrive resulting in denitration of GTN to GDN and GMN (Goel et al., 1996).

1.5. Objectives

In this study, the feasibility of grasses in cleaning up of propellant contaminated soil was observed by performing hydroponic and soil experiments. Three native grasses, yellow nutsedge, Indian grass and big bluestem, were used in clean-up of 2,4-DNT, perchlorate and GTN and their uptake and transformation were assessed.

Objective 1: Determine rate of uptake and transformation of the contaminants by the three grasses.

Hydroponic experiments were performed in laboratory conditions by analyzing propellant removal from contaminated water by pre-grown grasses. Through hydroponic studies the contaminant interaction with soil was eliminated, the effect of bacterial transformation was minimized, and the plant chemical interaction isolated. Although soil studies are more realistic, hydroponic experiments were performed first because of difficulties with soil contamination aging, abiotic contaminant degradation by soil

organic matter, non-uniform contaminant distribution, and proper soil selection. The uptake and transformation of contaminants in grasses were determined by analyzing the change in hydroponic solution concentrations over time and analyzing the accumulation of contaminants in plant tissue after completion of the experiments.

Objective 2: Determine the fate of the three contaminants in soil supporting growth of the three grasses.

Soil studies were carried out by transplant experiments, germination experiments and surface contamination experiments. In transplant experiments, soil was contaminated by spiking and pre-grown plants were transplanted in them. The removal of contamination from the soil was observed by sampling, extracting, and analyzing soil periodically. Germination experiments were performed to observe seed sprouting in contaminated soil. Seeds of Indian grass and big bluestem, and corms of yellow nutsedge were sown in contaminated soil and the germination period was observed. In surface contamination experiments, the top soil of the plant was contaminated with crystals of 2,4-DNT and perchlorate. In the case of GTN, the plant was watered with GTN stock solution as GTN crystals were too explosive to use. This setup mimics contamination observed at firing ranges where unexploded or partially exploded shells are the contamination source. The uptake and transformation of the contaminants by the plants was determined by extracting and analyzing contaminants in soil and plant tissue after two months of growth.

The ability of grasses to detoxify and transform contaminants in soil was analyzed by performing these experiments. Hydroponic experiments were conducted to observe the interaction between grass and contaminant without any biochemical or abiotic

influences. This was later applied to soil systems to study the biochemical and abiotic influences in their interaction. The effectiveness of grass in cleaning up contaminated soil was important in applying phytoremediation on a large scale. The phyto-mechanisms involved in uptake and transformation were also studied. The mass of contaminant taken up by the plant and the effectiveness of the plant in degrading the contaminant and the rate of contaminant degradation were observed.

2. Materials and Methods

2.1. Reagents and Standards

The 2,4-DNT crystals (Avocado Research Chemicals, Ward Hill, MA) were dissolved in ultrapure water to make stock solutions. Ten milligrams of 2,4-DNT crystals were dissolved in 1 liter of ultrapure water by stirring for one week to obtain a stock solution of concentration 10 mg/L. The 2,4-DAT, 2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene were purchased from Acros Organics (Morris Plains, NJ) for use as analysis standards. Methanol (Fisher Scientific, Pittsburgh, PA) was filtered (0.45 μm) along with ultrapure water to degas the solution and was used as an eluent (35%/75% methanol/water) in high performance liquid chromatography (HPLC). Sodium azide (J.T. Baker Chemical Co., Phillipsburg, NJ) was used to kill microorganisms in the roots of grasses in the killed control. Acetonitrile (Fisher Scientific, Pittsburgh, PA) was used to extract 2,4-DNT and metabolites from soil and plant tissue. Acetone (Fisher Scientific, Pittsburgh, PA) was used in dissolving 2,4-DNT for spiked soil studies. Ammonium perchlorate crystals were dissolved in ultrapure water to make a stock solution. Degradation product sodium chloride was purchased from Fisher Scientific (Pittsburgh, PA) while sodium chlorite and sodium chlorate were purchased from Acros Organics (Morris Plains, NJ). Ammonium perchlorate crystals were dissolved in ultrapure water to obtain a stock solution containing perchlorate of concentration 10 mg/L. NaOH purchased from Fisher Scientific (Pittsburgh, PA), was diluted with ultrapure water to make an eluent of concentration 35 mM for ion chromatography (IC). Nitroglycerin of 10 mg/mL dissolved in methanol was purchased from Restek (Bellefonte, PA). Custom made glycerol dinitrate (GDN) and glycerol mononitrate (GMN) standards were

purchased from AccuStandard (New Haven, CT). Nitroglycerin was diluted with ultrapure water obtain a stock solution of concentration 10 mg/L for hydroponic studies and 100 mg/L for surface contamination experiments.

2.2. 2,4-DNT Analysis

2,4-DNT was analyzed by HPLC equipped with diode array detection. A BDS Hypersil C18 column (100x4 mm) (Thermo electron corporation, Waltham, MA) was used with an eluent of 35% / 75% methanol / water, at a flow rate of 1 mL/min and an injection volume of 20 µL integrated at 254 nm. Calibration curves were set up over concentrations ranging from 0.5 mg/L to 10 mg/L. Separation of 2,4-DNT, 2-amino-4-nitrotoluene, and 4-amino-2-nitrotoluene was obtained as shown in Figure 5, Figure 6, and Figure 7 with respective elution times of 9.4 min, 5.5 min, and 5.1 min.

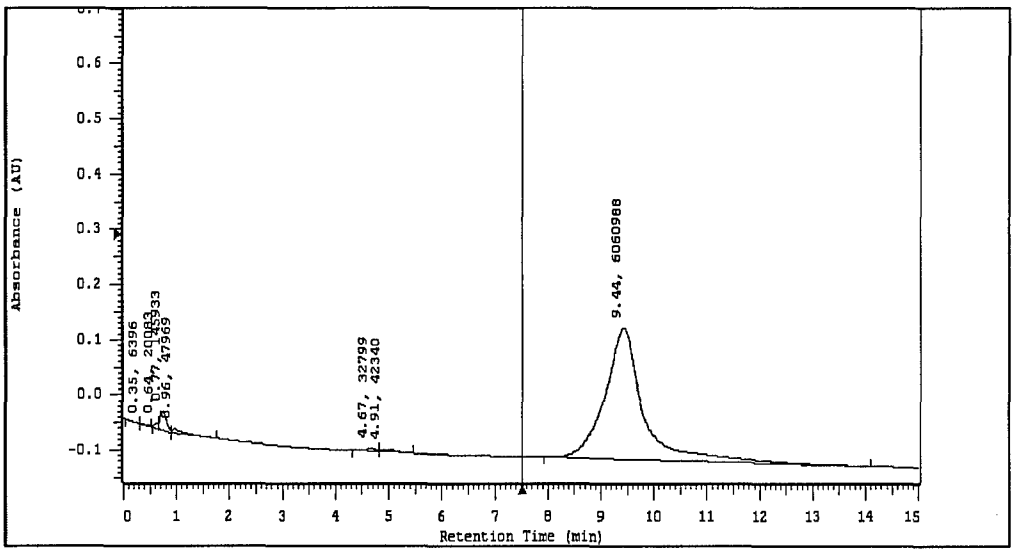

Figure 5: 2,4-DNT chromatograph

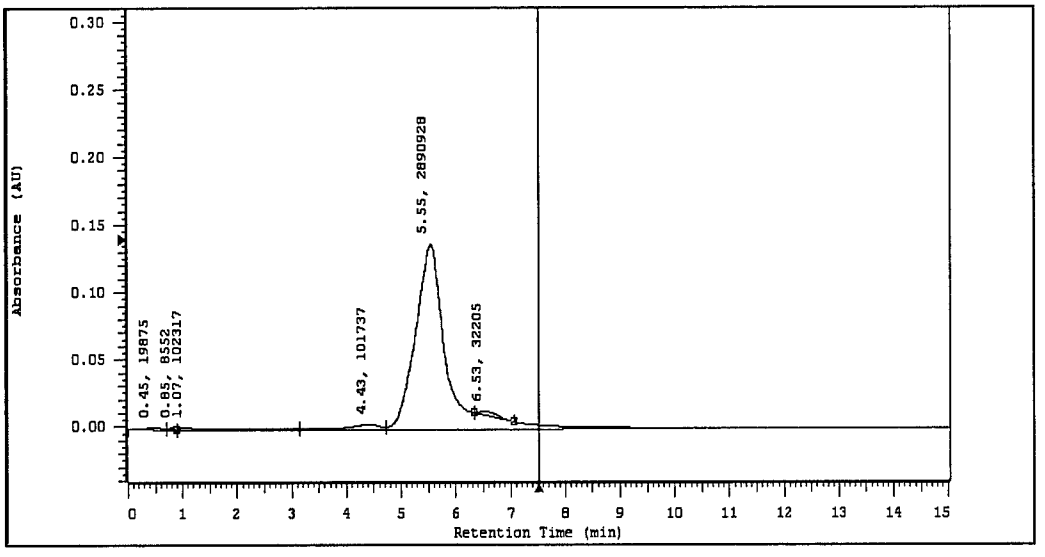

Figure 6: Chromatograph of 2-amino-4-nitrotoluene

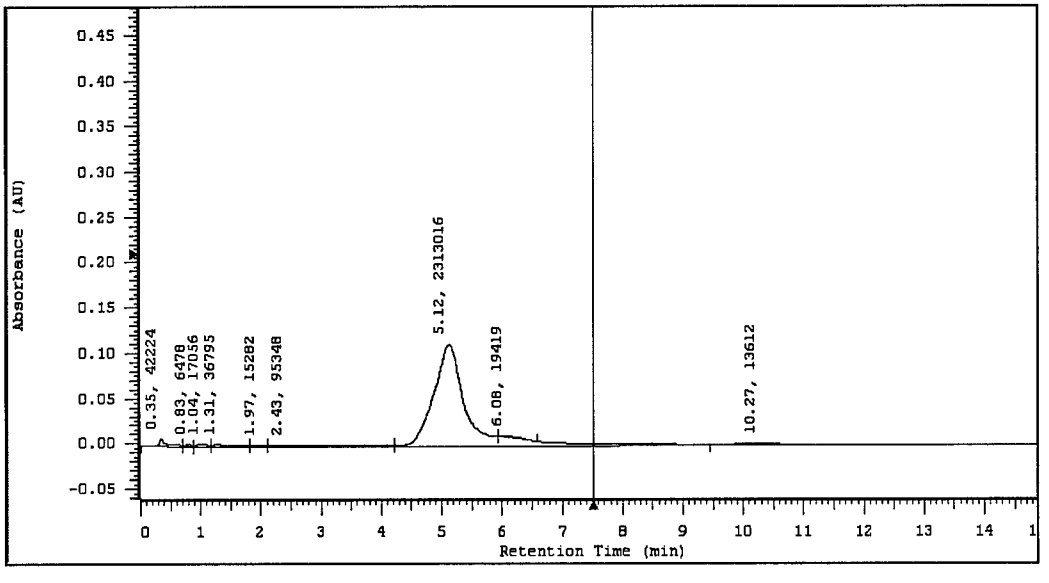

Figure 7: Chromatograph of 4-amino-2-nitrotoluene

To extract 2,4-DNT from roots, leaves, or soil, 1 to 2 g of chopped root, chopped leaf or soil was added to 5 mL acetonitrile in 40 mL gas tight sealed bottles (Figure 8). They were sonicated by a Branson 3210 ultrasonic system at 23 °C for 8 hours, centrifuged on a Biofuge A (American Scientific Products, Columbus, OH) for 5 min at 13,000 rpm, and the supernatant was analyzed by HPLC. Sub samples of leaf or root

tissues were oven dried at 105 °C overnight to obtain percent moisture, and concentrations were reported in mg 2,4-DNT per kg dry tissue (mg/kg).

$$\% \text{ Moisture} = \frac{(\text{Wet Weight} - \text{Dry Weight})}{\text{Wet Weight}} \times 100$$

Figure 8: Leaf and root extraction vials

2.3. Perchlorate Analysis

Perchlorate was analyzed on a Dionex ion chromatograph (IC 25, Sunnyvale, CA) equipped with an AS-40 automated sampler, and anion self regenerating suppressor (ASRS) Ultra II (4 mm). Perchlorate, chlorate, chlorite and chloride ions were separated using a Dionex IonPac AS 16 Analytical (4 x 250 mm) column, with guard Ion Pac AG-16 and 35 mM NaOH as eluent flowing through the system at 1 mL/min. Calibration curves were set up over concentrations ranging from 0.5 mg/L to 10 mg/L for perchlorate, chloride, chlorite and chlorate. The 35 mM NaOH eluent was kept

pressurized with helium gas at 8 psi (55 KPa). The injection loop and injection volume were 1 mL, and the run time was 20 minutes. Perchlorate eluted at 17.6 min, as shown in Figure 9. Chloride eluted at 3.8 min, chlorite eluted at 4.2 min and chlorate eluted at 4.8 min.

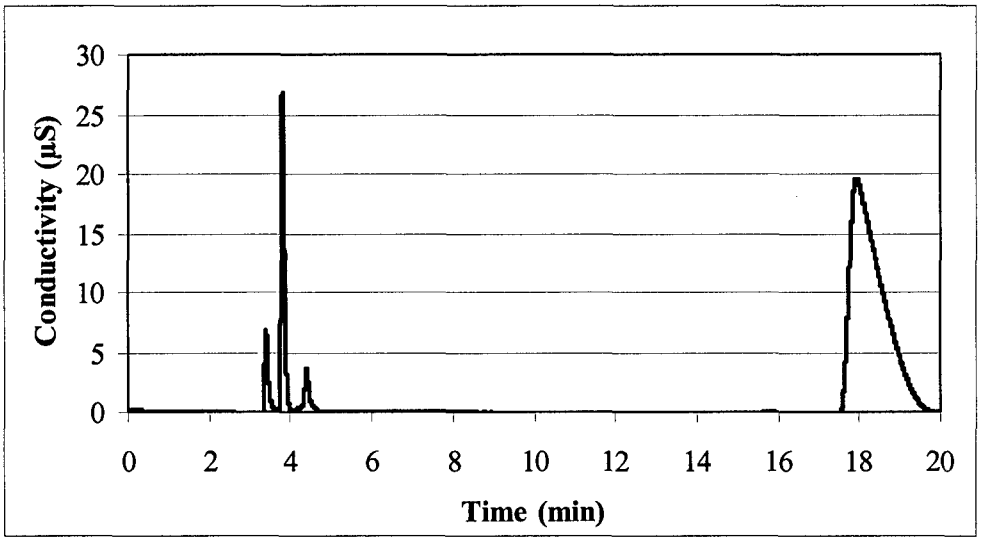

Figure 9: Chromatograph of perchlorate and metabolites showing elute times of 3.8 min for chloride, 4.2 min for chlorite and 4.8 min for chlorate, and 17.6 min for perchlorate

Perchlorate was extracted by boiling 0.5 to 2.5 g of chopped root, or leaf along with 45 mL ultrapure water on a hot plate for an hour. The solution was cooled and analyzed by IC for perchlorate and its breakdown products (Tan et al., 2006). Sub samples of leaf or root tissues were oven dried at 105 °C for 8 hours to obtain percent moisture, and concentrations were reported in mg perchlorate per kg dry tissue (mg/kg). Soil extraction was the same as 2,4-DNT and GTN but 35 mM NaOH was used instead of acetonitrile.

2.4. Nitroglycerin Analysis

Nitroglycerin was analyzed by HPLC using the same method as described for 2,4-DNT. Calibration curves were set up over concentrations ranging from 0.5 mg/L to 10 mg/L. Nitroglycerin was eluted at 8.16 min, GDN eluted at 2.7 min, and GMN eluted at 2.4 min (Figure 10 and Figure 11). The two different GDN and GMN isomers could not be resolved by this method.

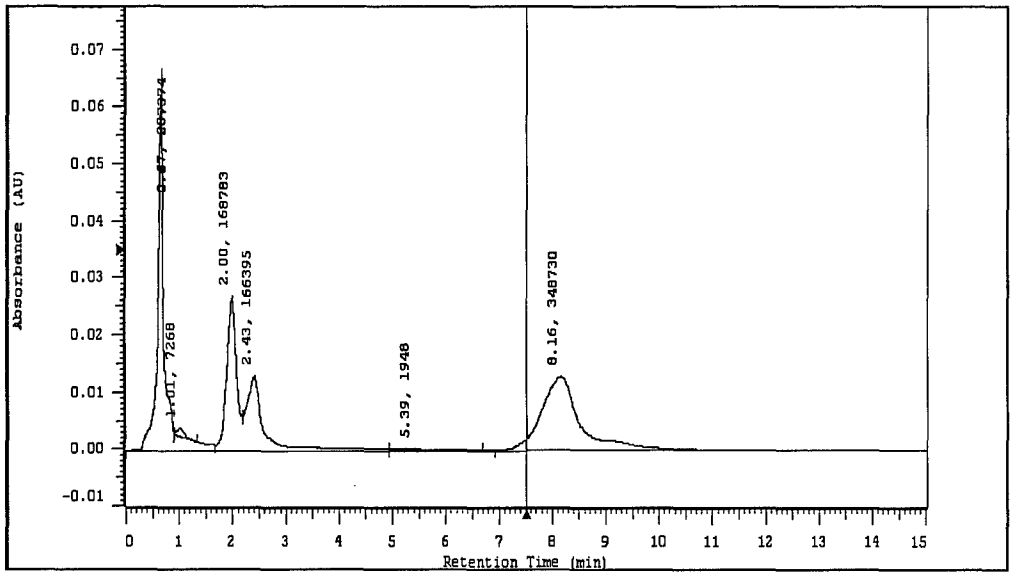

Figure 10: Nitroglycerin chromatograph

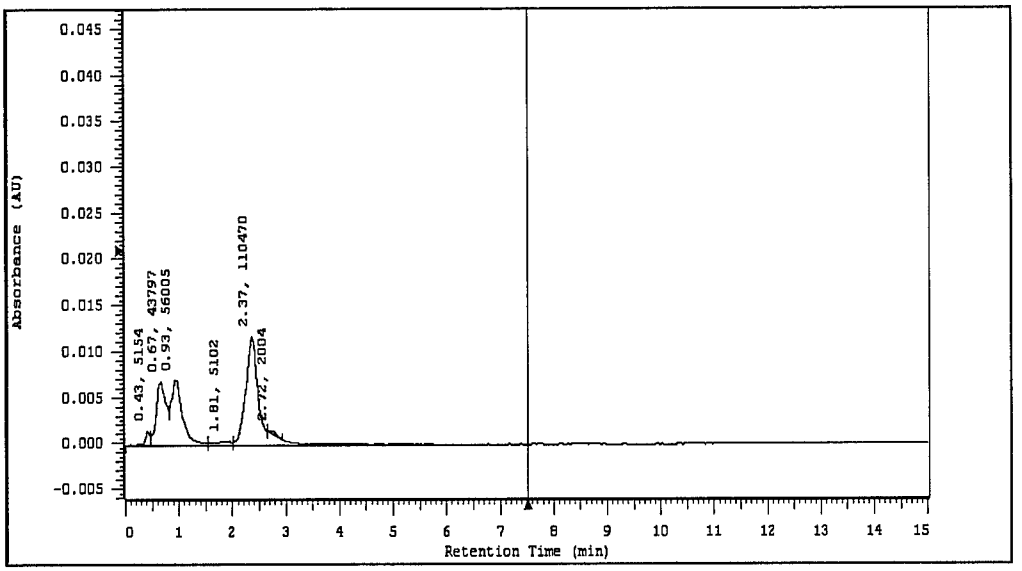

Figure 11: Chromatograph of GDN

Nitroglycerin was extracted from roots and leaves by the same method described for 2,4-DNT.

2.5. Hydroponic Experiments

Three native grasses chosen for this experiment were yellow nutsedge (*Cyperus escalantus*), Indian grass (*Sorghastrum nutans*) and big bluestem (*Andropogon gerardii*). Yellow nutsedge was started from corms while Indian grass and big bluestem were started from seeds. They were grown in potting soil 3 months prior to the start of experiments, under lights, on a 12 hour on/off schedule.

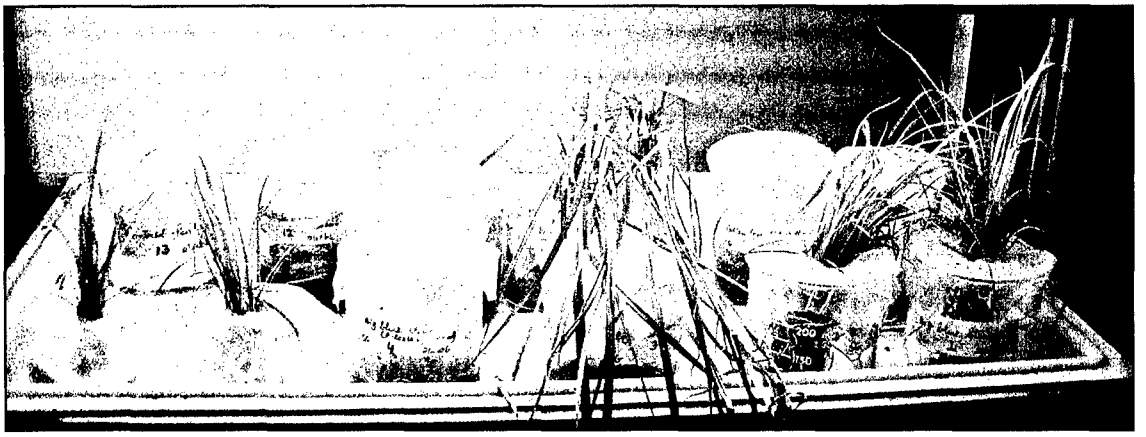

Figure 12: Set up of hydroponic studies

5 to 15 g grass/L of stock solution was added to clean, glass beakers and kept under grow lights on a 12 hour on/off schedule. The plant roots were carefully separated from soil and washed with tap water. The beakers were covered with parafilm to minimize evaporation of stock solution. All beakers were prepared in duplicate for perchlorate and GTN and in triplicate for 2,4-DNT. For 2,4-DNT and GTN, 0.75 mL of water samples were collected every 12 hours, for up to 60 hours, mixed with 0.75 mL of methanol, and analyzed by HPLC. For perchlorate, 5mL of water samples were collected every 24 hours, for up to one week and were analyzed by IC. There were three controls used for each contaminant; no grass control, frozen root control and killed control with frozen roots. Roots were snipped from one set of washed plants and frozen overnight to kill the tissue. Sodium azide at 1 mg/L was added to frozen roots of the killed control to inhibit bacterial activity.

Contaminant removal curves were analyzed to determine kinetic rate constants. Two removal models were tested; zero order $\frac{dC}{dt} = k_1$, *or* $C = -k_1 + C_0$ and first order $\frac{dC}{dt} = k_2 C$ *or* $C = C_0 e^{-k_2 t}$. These regressions were performed using MS Excel's trendline

feature. R^2 values indicating goodness-of-fit were also reported (better fits have R^2 values closer to 1.0).

2.6. Transplant Experiments

50 mg/kg of 2,4-DNT were dissolved in 5 mL of acetone and added to 4 kg of WES soil. WES soil, sandy silt with low organic content and sieved for uniformity, was collected from the US Army Corps Waterways Experiment Station in Vicksburg, MS. This was mixed under the hood everyday for a month to ensure complete mixing of 2,4-DNT and soil and complete acetone evaporation.

For the control, 4 kg of WES soil was sterilized in an autoclave repeatedly for three days to kill spore forming organisms. It was spiked with 2,4-DNT from the previously described acetone mixture and was mixed as before. Soil was analyzed periodically in both sterilized and live soil during mixing over a period of 1 month.

Known amounts of live and sterile soil were added to 250 mL beakers and pre-grown plants (grown over a period of two months) were weighed and transplanted into them. Each type of soil had one unplanted control. The transplanted plants and the control were watered every day to field capacity. All beakers were prepared in duplicate. Soil samples of were collected every three days for a period of 12 days by coring vertically down through the depth of the soil near the plant with a 1/2" diameter corer. Cored holes were marked with toothpicks to prevent sampling the same location twice.

2.7. Germination Experiments

Seeds of big bluestem and Indian grass and corms of yellow nutsedge were planted in beakers with live and sterile soil to measure germination rates. Seeds in clean soil were used as controls. Equal number of seeds was placed in each beaker; 10 for big

bluestem and Indian grass, and 6 for yellow nutsedge. All beakers were prepared in duplicate.

2.8. Surface Contamination Experiments

Yellow nutsedge (*Cyperus escalantus*) and Indian grass (*Sorghastrum nutans*) were grown in beakers from corm and seed two months prior to the start of experiments, under grow lights on 12 hour on/off schedule. Glass beads were placed in the bottom of beakers to increase aeration for plants. Local Athens soil was used and was sieved to remove rocks and other large particles. The soil was a mixture of sand and clay in almost equal proportions. After two months of growth, beakers were contaminated with 500 mg of 2,4-DNT or ammonium perchlorate crystals. Crystals were placed on the top portion of the soil and the beakers were watered with a known amount of water to field capacity (Figure 13). Because GTN crystals are highly explosive, GTN stock solution of concentration 100 mg/L was prepared prior to the start of experiments and plants were watered with this solution for the duration of the experiment. All beakers were prepared in duplicate for each contaminant. Two controls were used for this experiment for each contaminant; unplanted live soil and unplanted sterile soil. Soil was sterilized by autoclaving it on three consecutive days to kill spore forming bacteria. At the end of 2 weeks, 2,4-DNT, perchlorate and GTN was extracted from the top soil, bottom soil, glass beads, and from leaves and roots of the plants.

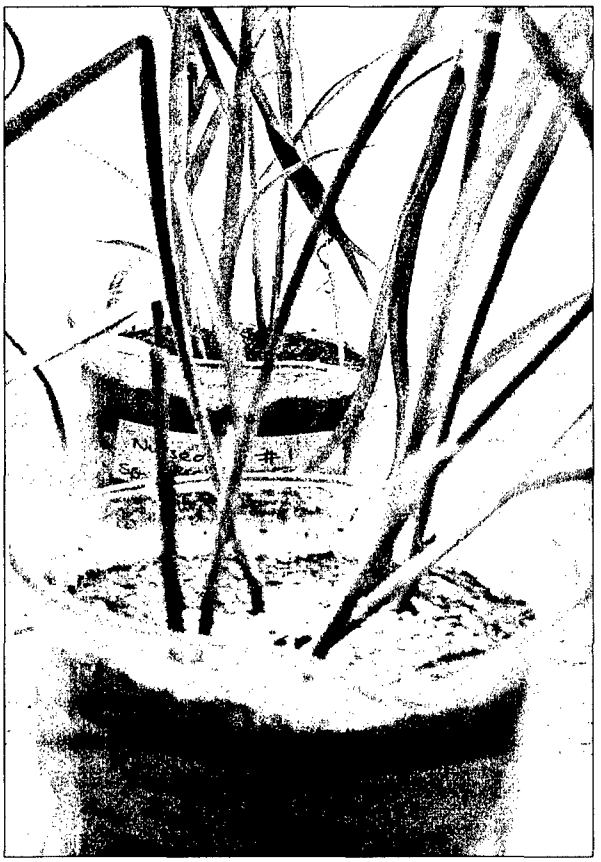

Figure 13: Setup of surface contamination experiments. Crystals are apparent on the surface of the soil.

3. Results and Discussion

3.1. Hydroponic Experiments

3.1.1. 2,4-DNT

Yellow nutsedge (*Cyperus escalantus*), Indian grass (*Sorghastrum nutans*) and big bluestem (*Andropogon gerardii*) were used in hydroponic studies to determine the uptake and transformation of 2,4-DNT, perchlorate and GTN. In 2,4-DNT hydroponic experiments, water samples were collected every 12 hours and analyzed by HPLC. A significant decrease in 2,4-DNT concentration was seen in the three grasses.

With big bluestem, 2,4-DNT concentrations were reduced to less than 1 mg/L in the live plants, frozen roots control, and killed control within 36 hours from the start of the experiment indicating rapid removal of 2,4-DNT from the stock solution (Figure 14). Concentrations in the no plant control remained virtually constant. No 2,4-DNT degradation products were detected in any of the trials.

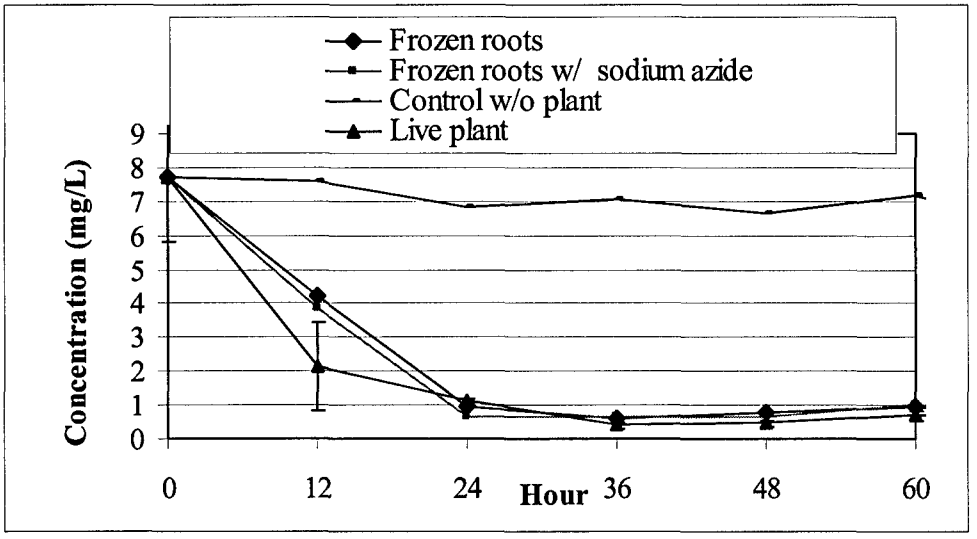

Figure 14: Concentration of 2,4-DNT in big bluestem in hydroponic studies. For the live plant average values of three experiments are shown with error bars designating one standard deviation.

In Indian grass, the live plant was effective in degrading 2,4-DNT at a faster rate when compared to big bluestem (Figure 15). The concentration was reduced to less than 1 mg/L within 24 hours in the live plant after which the removal stopped. The concentration profile for the frozen root control and killed control of Indian grass was similar to that of big bluestem. 2,4-DNT was degraded rapidly in the frozen root control and killed control with concentrations less than 1 mg/L at the end of 52 hours. The uptake in the killed and frozen root controls was somewhat slower than in the live plant. The unplanted control did not see any significant change in concentration and remained constant throughout the experiment. No 2,4-DNT degradation products were detected in any of the trials.

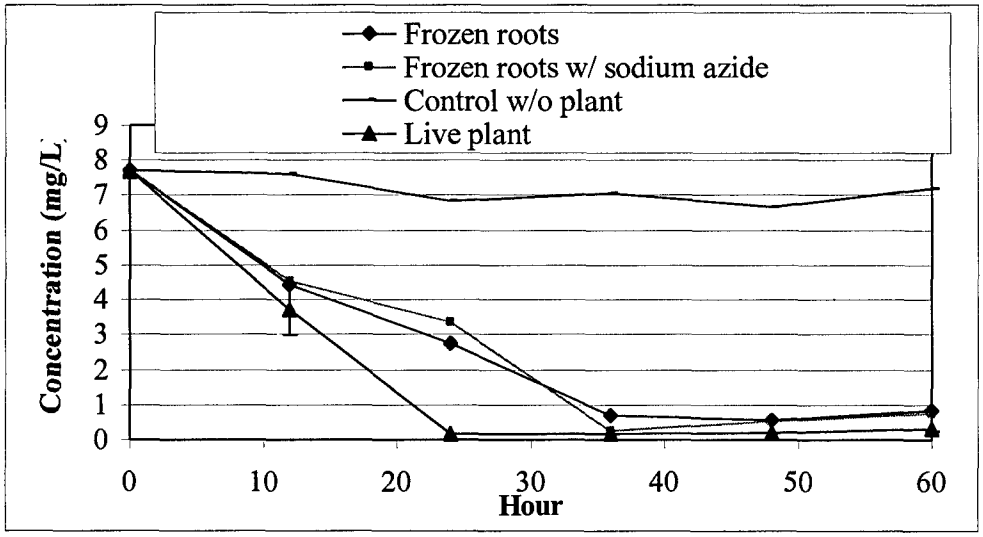

Figure 15: Concentration of 2,4-DNT in Indian grass in hydroponic studies. For the live plant average values of three experiments are shown with error bars designating one standard deviation.

From Figure 16, it is evident that 2,4-DNT was not effectively transformed in yellow nutsedge. At the end of 60 hours, the concentration in solution decreased from 7.8 mg/L to 5.3 mg/L. The slight increase in concentration towards the end of the experiment

was likely due to concentration of 2,4-DNT as water was removed by evapotranspiration. The frozen root control and killed control had significantly faster removal rates than the live plant. The unplanted control did not see any significant change in concentration, and no 2,4-DNT degradation products were detected in any of the trials.

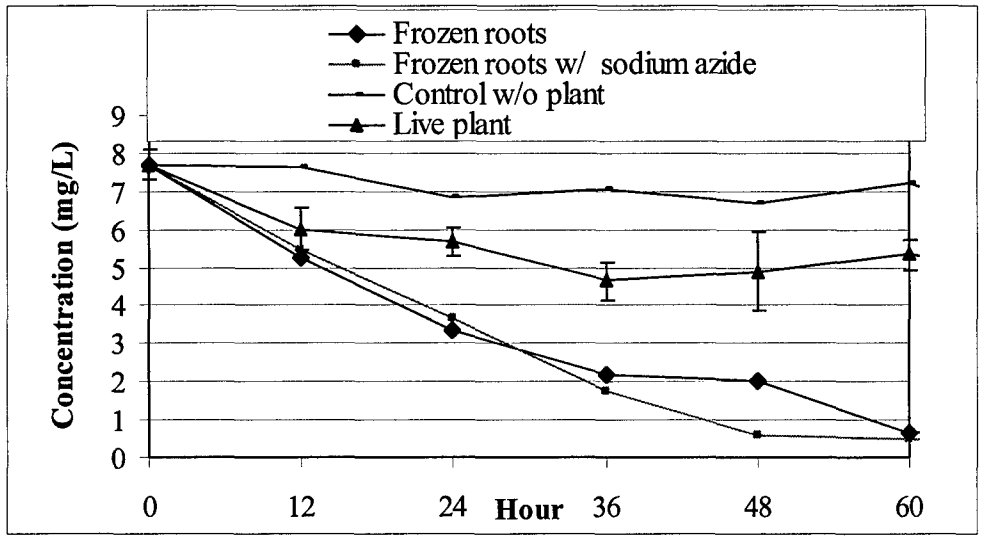

Figure 16: Concentration of 2,4-DNT in yellow nutsedge in hydroponic studies. For the live plant average values of three experiments are shown with error bars designating one standard deviation.

Linear and exponential fits for big bluestem, Indian grass and yellow nutsedge are given in Figure 17, Figure 18, and Figure 19. For many of the curves 2,4-DNT removal stopped for the last few data points. Because the first order model assumes continuous removal, these final points were removed from the data set before fitting. This not only gave more realistic removal rates but also better R^2 values for the three grasses. Linear and exponential curve fits were plotted for live plant, frozen root and killed control of the three grasses.

Zero (linear) and first (exponential) order removal rates for big bluestem, Indian grass and yellow nutsedge are shown in Table 2. Indian grass and big bluestem were found to have significantly higher zero and first order removal rates in the live plants, frozen roots and killed controls than yellow nutsedge. For all the grasses, the frozen root and killed controls performed similarly. For big bluestem removal rates for the live plants, frozen roots, and killed controls were indistinguishable. For Indian grass removal rates for the live plant were significantly higher than for the frozen root and killed controls, while for yellow nutsedge removal rates were lower than for the frozen root and killed controls. R^2 values indicate that the first order model provided a slightly better fit to the data than the zero order model for most of the experiments.

The four different treatments in this experiment were designed to isolate different mechanism of 2,4-DNT removal. In the no plant control, loss of 2,4-DNT by hydrolysis reactions, sorption to glassware, and volatilization were measured. As expected, losses by these mechanisms were negligible. The two frozen root controls were frozen overnight to kill the plant tissue. These treatments measure passive sorption to the roots in comparison to active uptake observed in the live plants. For all of the plants, removal from solution was significant in the frozen root controls, with removal rates only slightly higher in live Indian grass and live big bluestem, while being significantly lower in live yellow nutsedge. Because 2,4-DNT is hydrophobic (log K_{ow} = 1.98), it was not surprising that passive sorption would be significant, and in fact dominate 2,4-DNT removal. The yellow nutsedge results were surprising, because they indicate that the live plant was somehow preventing sorption into the roots presumably by actively pumping 2,4-DNT back into the solution. Finally, the killed control contained frozen roots and a toxic

concentration of sodium azide. This compound disrupts the respiratory enzymes in bacteria effectively preventing any microbial activity in the solution. In all three plants, there was no significant difference between frozen roots and frozen roots with sodium azide, indicating bacteria did not play a role in 2,4-DNT removal.

Table 2: Rate constants for 2,4-DNT

Grass		Rate constant ($mg\ L^{-1}\ hr^{-1}$) Linear	R^2	Rate constant (hr^{-1}) Exponential	R^2
Big bluestem	live plant	0.191	0.795	0.079	0.985
	frozen root	0.205	0.912	0.076	0.951
	killed control	0.203	0.882	0.076	0.891
Indian grass	live plant	0.218	0.883	0.121	0.856
	frozen root	0.190	0.978	0.065	0.931
	killed control	0.196	0.973	0.088	0.804
Yellow nutsedge	live plant	0.080	0.935	0.013	0.954
	frozen root	0.155	0.976	0.036	0.999
	killed control	0.164	0.998	0.041	0.964

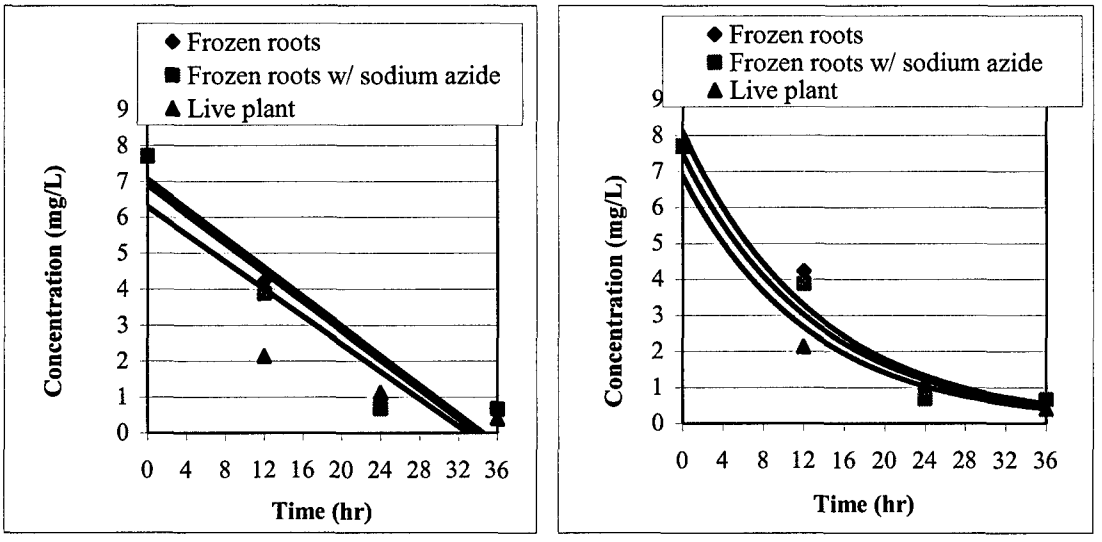

Figure 17: Linear and Exponential curve fits for big bluestem in hydroponic studies

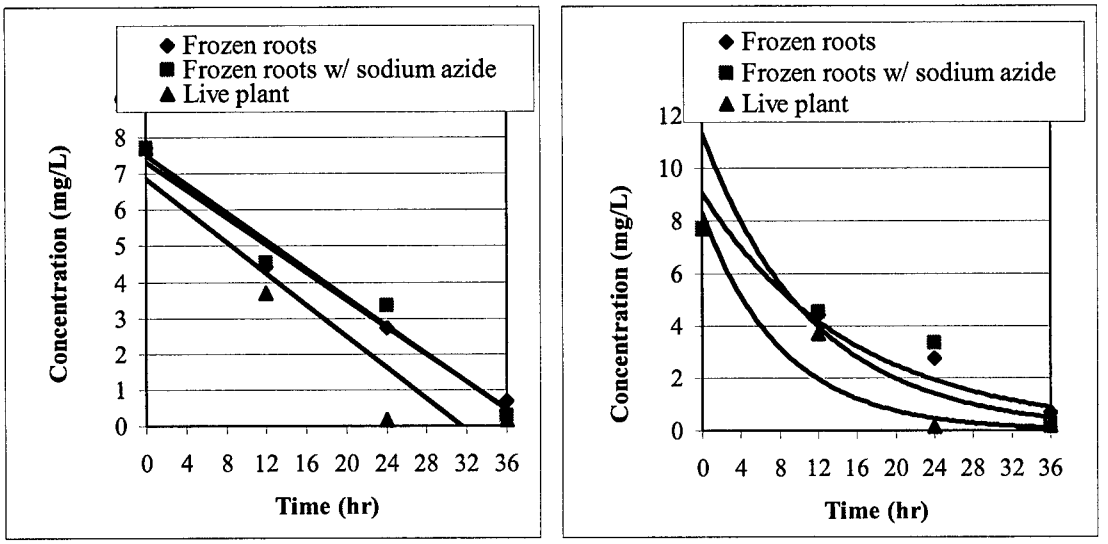

Figure 18: Linear and exponential curve fits for Indian grass in hydroponic studies

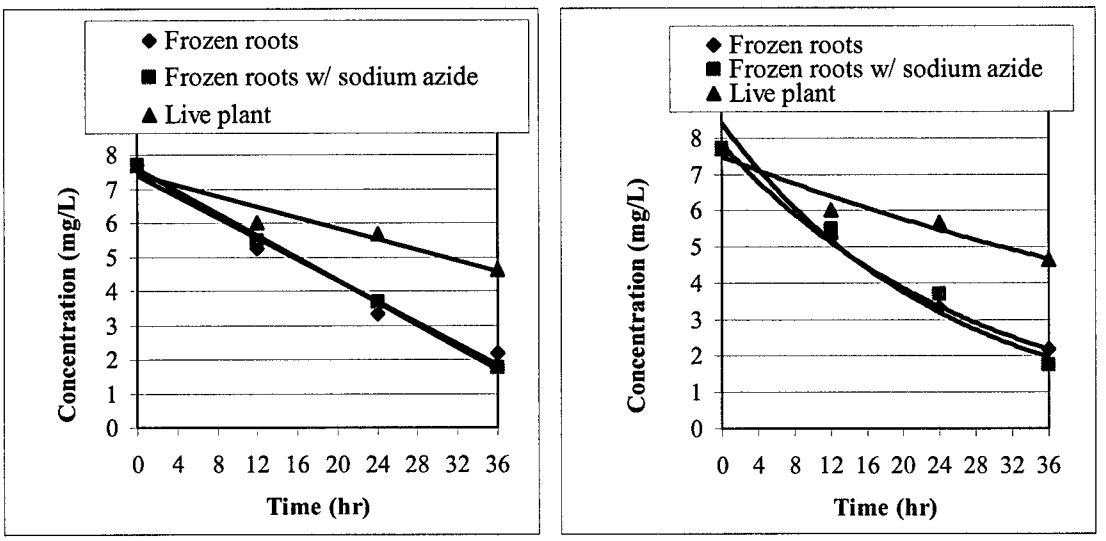

Figure 19: Linear and exponential curve fits for yellow nutsedge in hydroponic studies

After completion of the hydroponic experiments (60 hours), plants were separated into leaves and roots, chopped, extracted, and analyzed by HPLC. Highest 2,4-DNT concentrations were measured in yellow nutsedge which had 30.4 $\pm$ 9.2 mg/kg in leaves and 107.7 mg/kg in roots (Table 3). Large amounts of accumulation were also found in the frozen root and killed control of yellow nutsedge. For all of the plants, accumulation

was found to be greater in the roots than in the leaves. In big bluestem, the ratio of root to leaf was 2.25:1; in Indian grass it was 3.3:1, and 3.5:1 in yellow nutsedge. Big bluestem had larger amounts of 2,4-DNT accumulated in the roots in the frozen and killed controls than in the live roots, possibly indicating the presence of a plant enzyme degrading 2,4-DNT. Traces of 2-amino-4-nitrotoluene were found in the live plant, frozen roots, and killed control for each of the plants, but concentrations were below 1 mg/kg. This indicates that 2,4-DNT transformation was occurring in the plants. Presence of 2,4-DNT in the leaf tissue indicates that 2,4-DNT was actively taken up by the plants, although concentrations were significantly lower than in the roots that were also subjected to sorption.

Table 3: 2,4-DNT uptake in grasses in hydroponic studies

Grass	Concentration of 2,4-DNT mg DNT/kg grass (dry weight)			
	Leaves[a]	Roots	Frozen roots	Killed control
Yellow Nutsedge	30.4 ± 9.2	107.7	106.5	83
Indian grass	23.1	76.7	77.8	84.5
Big bluestem	25.9	58.1	84.2	70.2

Note:
[a] Although experiments were carried out in triplicate, only yellow nutsedge had enough biomass to carry out triplicate extraction of leaves. As such, the average of 3 samples ± one standard deviation is reported. For the remaining plants and all of the roots, biomass from the triplicate experiments was combined to perform a single extraction and analysis.

The fate of 2,4-DNT in the plants was calculated by performing mass balance calculations. The mass of 2,4-DNT found in the leaves and roots was divided by the initial mass of 2,4-DNT used in the hydroponic solution to obtain the percent accumulation of 2,4-DNT. 26% accumulation of 2,4-DNT was seen in yellow nutsedge

(leaf and roots combined), 23% in Indian grass and 12% in big bluestem (Figure 20). Yellow nutsedge removal from the solution was 25% of the initially added 2,4-DNT mass (Figure 16) matching the accumulation found in the root and leaf tissues. This indicates yellow nutsedge took up 2,4-DNT but little degradation occurred. Significant accumulation was also seen in frozen root and killed controls. 27% uptake was seen in the frozen roots controls of yellow nutsedge, 21% in big bluestem and 19% in Indian grass, while 21%, 17.5% and 21% were seen in the killed controls.

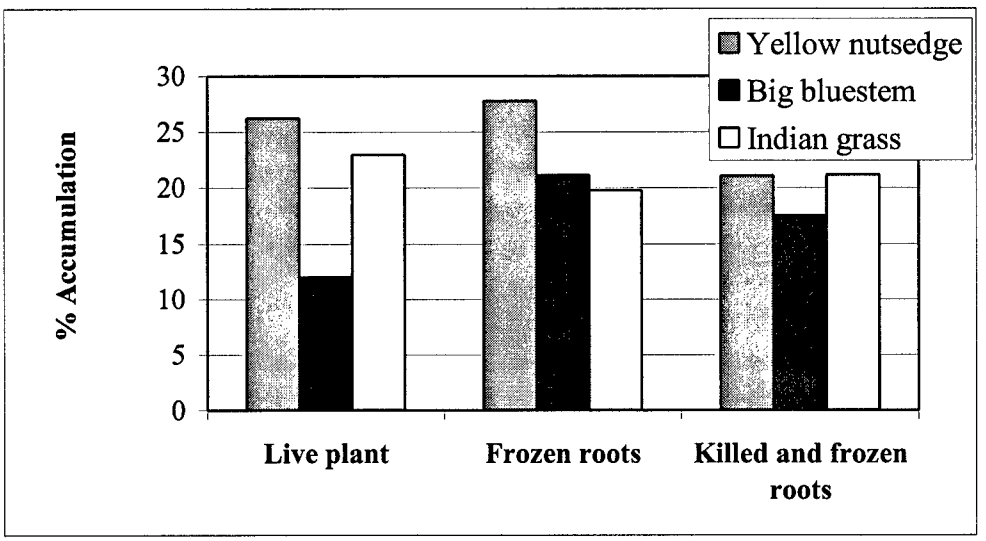

Figure 20: Accumulation of 2,4-DNT in plant tissue

From Table 4, the mass balance values are reported and the percent of 2,4-DNT degraded estimated. The mass of 2,4-DNT that was not accounted for in the plant or remaining solution was assumed to be either transformed by bacteria or plant enzymes into another chemical. It is evident that there was significant 2,4-DNT degradation in most of the treatments. Big bluestem accumulated considerably less 2,4-DNT when compared to yellow nutsedge and Indian grass, while removing 86% of the initially added 2,4-DNT. This is a good indication that significant 2,4-DNT degradation occurred in big bluestem. Also, these results indicate that 2,4-DNT uptake in big bluestem was passive,

and given the high K_{ow} for 2,4-DNT, it was likely dominated by abiotic sorption. In Indian grass, 76.2% of the initially added 2,4-DNT was degraded by the live plant. Significant accumulation was seen in live plant, frozen roots and killed control indicating that the uptake in Indian grass was also largely passive with only a minor increase in uptake rate with the live plant. Percentage degraded for big bluestem and Indian grass were not significantly different among the live plants, frozen roots, and killed controls. Yellow nutsedge was slow in removing 2,4-DNT from solution but was effective in accumulating 2,4-DNT in roots and leaves of the plant. This suggests that yellow nutsedge has some active mechanism to limit 2,4-DNT uptake. Abiotic sorption in the dead roots and killed control was evident due to 2,4-DNT accumulation in yellow nutsedge (log K_{ow} = 1.98). Percent degradation was lowest in yellow nutsedge, even lower than the frozen root and killed controls.

Table 4: Mass balance of 2,4-DNT in hydroponic studies

Plant		% of 2,4-DNT				
		Leaves	Roots	Water[a]	Total	% mass degraded
Yellow nutsedge	live plant	3.8	22.4	21.6 ± 18.6	47.8 ± 18.6	52.2 ± 18.6
	frozen roots	-----	27.8	0	27.8	72.2
	killed control	-----	21.0	0	21.0	79.0
Indian grass	live plant	4.4	18.5	0.9 ± 1	23.8 ± 1	76.2 ± 1
	frozen roots	-----	19.7	0	19.7	80.3
	killed control	-----	21.1	0	21.1	78.9
Big bluestem	live plant	3.2	8.8	1.9 ± 2.1	13.9 ± 2.1	86.1 ± 2.1
	frozen roots	-----	21.1	0	21.1	78.9
	killed control	-----	17.5	0	17.5	82.5

Note:
[b] The amount of water left in the beakers at the end of the experiment was not measured, and hence, the plants were assumed to be transpiring the same amount of water as in the GTN hydroponics experiment.

The high percentage of 2,4-DNT degraded in the frozen controls hint at the primary degradation mechanism. Freezing kills the tissue and prevents production of new enzymes and the possibility that 2,4-DNT was degraded by plant enzymes. Further, the killed control prevents bacterial activity and since there was no significant difference in degradation between frozen controls and killed controls, bacteria did not play a role. Also only trace concentrations of degradation products were detected. Nitroaromatic compounds have been shown to covalently bind to organic matter, effectively becoming part of the organic macromolecules (Achtnich et al., 2000). This likely explains why such high percent degradations were observed for killed controls and only trace amounts of degradation products were detected. Only in live big bluestem is the percent degraded significantly greater than the frozen root controls, indicating this species may have effective enzymes for 2,4-DNT degradation while yellow nutsedge and Indian grass do not. In fact, yellow nutsedge appears to have a mechanism to minimize 2,4-DNT transformation because the percent degraded in live plants was significantly below the amount for frozen controls.

Literature reported results for 2,4-DNT hydroponic studies have shown rapid decrease in 2,4-DNT concentration in live plants of yellow nutsedge with complete removal in less than 2 d (Riefler and Medina, 2006). The removal rates where 0.19 mg L^{-1} hr^{-1} for a zero order model and 0.04 hr^{-1} for a first order model. Comparing these removal rates to the rates obtained in this study (0.08 mg L^{-1} hr^{-1} and 0.013 hr^{-1}), it is evident that 2,4-DNT removal in the live plant of yellow nutsedge in this study was proceeding at a slow rate. Also, 2,4-DNT accumulation in the live plant of yellow nutsedge was more (26%) and only traces of degradation products were found during

extraction when compared to literature reported results which had lesser accumulation (11%) but increased concentrations of degradation products (Riefler and Medina, 2006). This indicates that some active mechanism in yellow nutsedge was limiting 2,4-DNT uptake and transformation in the live plant. Sorption of 2,4-DNT to dead roots was evident in previous study from significant uptake in dead roots of yellow nutsedge (Riefler and Medina, 2006). In other work, the degradation rates of TNT and 2,4-DNT were found to be comparable (Brannon and Pennington, 2002). 87.5% TNT removal in 2 d was observed in hydroponic experiments (Yoon J.M. et al., 2006). This was similar to 2,4-DNT removal in this study where nearly 90% degradation occurred at the end of 2 d, with big bluestem and Indian grass.

3.1.2. Perchlorate

In perchlorate hydroponic experiments, plant uptake was minimal. In fact while transpiring water, the plants selectively excluded perchlorate, which is evident from the increase in concentration of perchlorate in the hydroponic solutions. In big bluestem, perchlorate concentration increased from 8.6 mg/L to 16 mg/L (Figure 21). However, slow but steady decrease in perchlorate concentration was seen in the frozen root control. At the end of 5 days, the concentration of perchlorate in the frozen root control was 5 mg/L. The concentrations in the killed control and unplanted control remained virtually constant throughout the experiment. No perchlorate degradation products were detected in the solutions of any of the treatments.

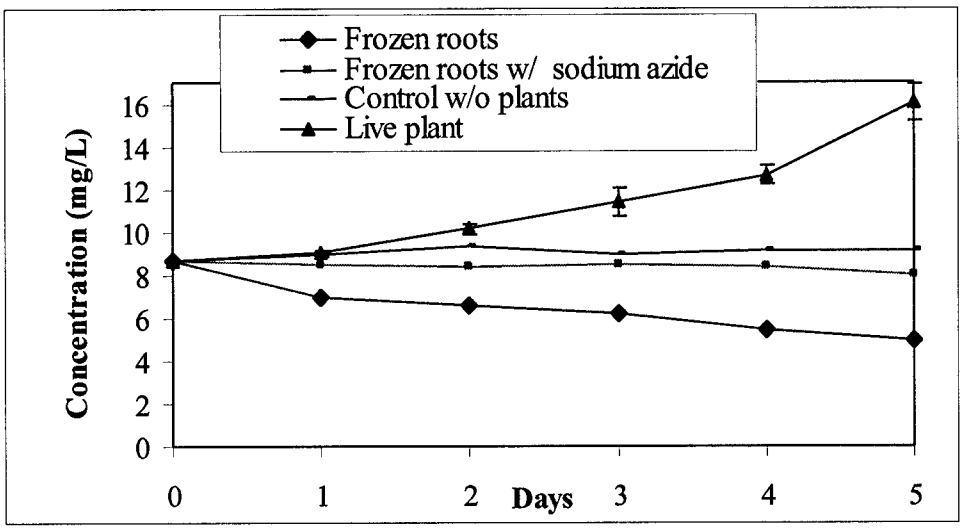

Figure 21: Concentration of perchlorate in big bluestem in hydroponic studies

Less change in perchlorate concentrations were seen in Indian grass than big bluestem and yellow nutsedge (Figure 22). Indian grass also transpired less water compared to yellow nutsedge and big bluestem, which accounts for its slower rate of perchlorate accumulation. The amount of water transpired by Indian grass over 5 days was 38.2 $\pm$ 5 mL when compared to big bluestem and yellow nutsedge that transpired 82 $\pm$ 2 mL and 122 mL of water, respectively. There was an increase in the concentration of perchlorate in the water solution from 8 mg/L to 11 mg/L. The concentrations of perchlorate in the frozen root control, the control with no plants, and the killed frozen root control all remained relatively constant over the length of the experiment. No degradation products were detected in any of the solutions.

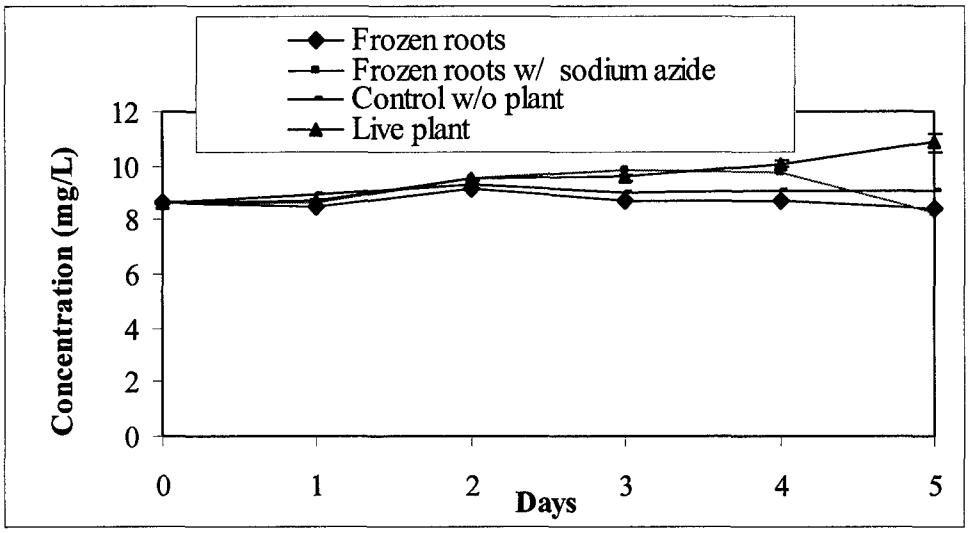

Figure 22: Concentration of perchlorate in Indian grass in hydroponic studies

In yellow nutsedge there was an increase in concentration from 8.6 mg/L to 14 mg/L (Figure 23). The concentration profiles for yellow nutsedge trials were similar to those of big bluestem. Degradation in frozen roots was taking place at similar rates as that of big bluestem. Perchlorate concentration decreased from 8.6 mg/L to 4.8 mg/L in 5 days. The plant was found to be transpiring more water than Indian grass and big bluestem (122 mL). No significant change in concentration was seen in the unplanted control and killed control, and degradation products were not detected in any of the solutions.

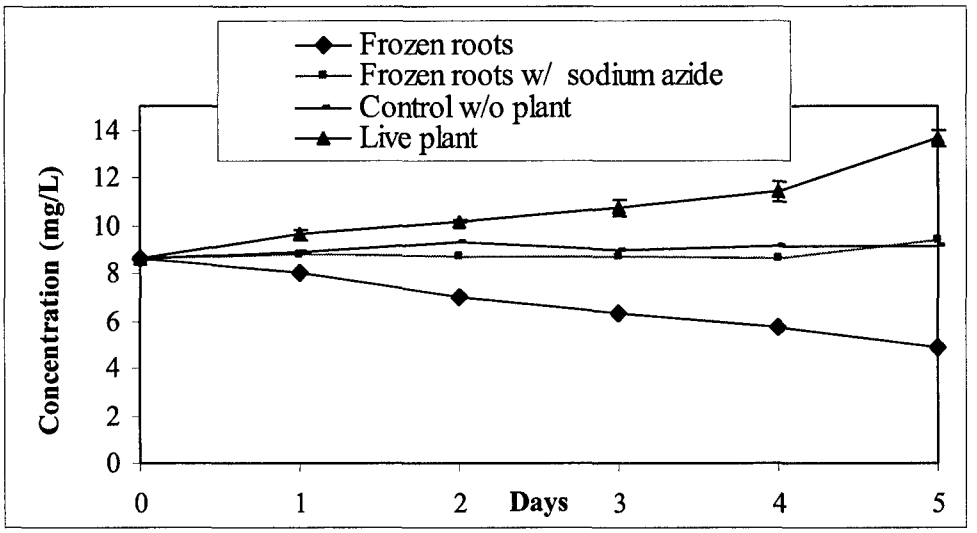

Figure 23: Concentration of perchlorate in yellow nutsedge in hydroponic studies

The zero and first order removal rates for perchlorate are given in Table 5 and the curve fits are shown in Figure 24, Figure 25, and Figure 26. Since the concentration in the live plants of the grasses were transpiring water and leaving perchlorate behind in the solution, there was a significant increase in perchlorate concentration in live plants. Hence, rates were only computed for declining curves. Frozen big bluestem and yellow nutsedge roots had more rapid removal rates than Indian grass indicating possible bacterial activity. Degradation rates of perchlorate in the killed controls were at extremely slow rates for all the plants.

In order to isolate the dominant mechanism involved in perchlorate removal, four different treatments were setup up for this experiment. In the unplanted control, perchlorate loss due to sorption to glassware, volatilization, evaporation, and hydrolysis reactions were assessed. As expected, perchlorate loss in solution was negligible and was not influenced by these factors. Loss of perchlorate due to sorption or bacterial degradation was measured by freezing roots overnight and determining the perchlorate

uptake to dead roots. Loss of perchlorate in frozen root controls in all the grasses was significant from the decrease in concentration. Removal rates for big bluestem and yellow nutsedge were higher than for Indian grass. Indian grass behaved differently than big bluestem and yellow nutsedge with extremely low removal rates in both frozen and killed controls and less transpiration in the live plants. In the killed control of all the grasses, perchlorate sorption to dead roots was insignificant which is not surprising due to its log K_{ow} value of -5.84. Further, perchlorate is hydrophilic, which also explains why perchlorate was not removed from the solution by sorption. In all the grasses there was a significant difference in removal rates between frozen root and killed controls indicating that the dominant mechanism in perchlorate removal was bacteria. The presence of bacteria in the frozen roots actively degraded perchlorate with higher removal rates in yellow nutsedge and big bluestem than in Indian grass.

Table 5: Rate constants for perchlorate in hydroponic studies

Grass		Rate constant (mg L^{-1} hr^{-1})	R^2	Rate constant (hr^{-1})	R^2
		Linear		**Exponential**	
Big bluestem	frozen root	0.0275	0.932	0.00425	0.962
	killed control	0.00369	0.745	0.000442	0.740
Indian grass	frozen root	0.00220	0.022	0.000133	0.038
	killed control	0.00114	0.036	0.000217	0.018
Yellow nutsedge	frozen root	0.03164	0.993	0.000442	0.988
	killed control	0.00402	0.333	0.000417	0.329

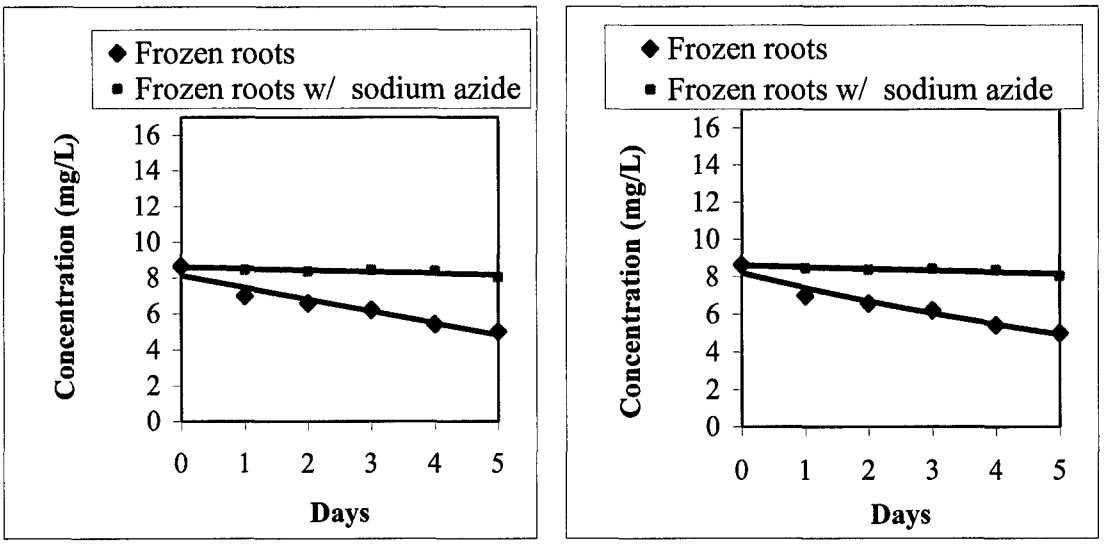

Figure 24: Linear and exponential curve fits for big bluestem in hydroponic studies

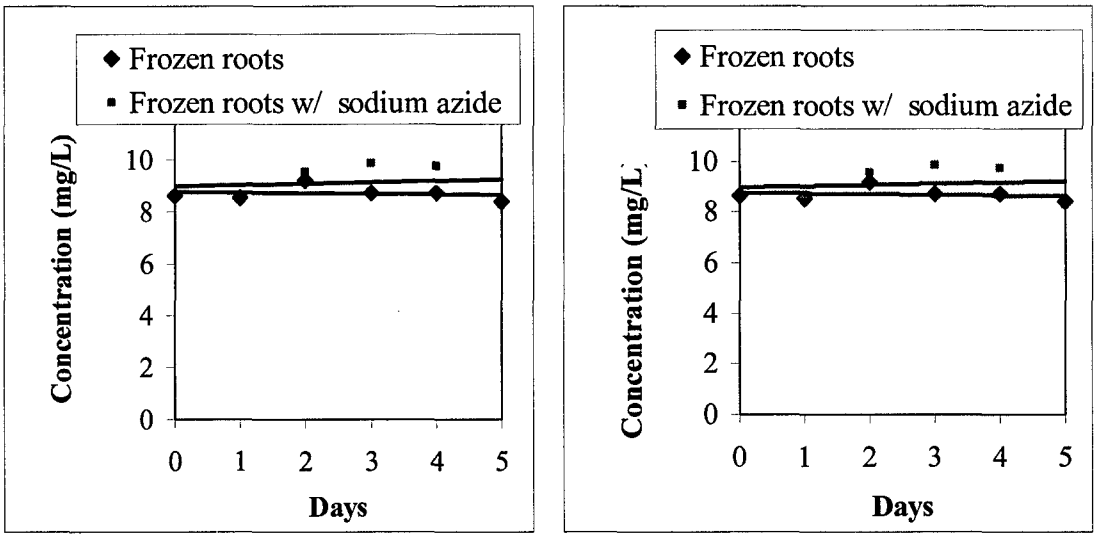

Figure 25: Linear and exponential curve fits for Indian grass in hydroponic studies

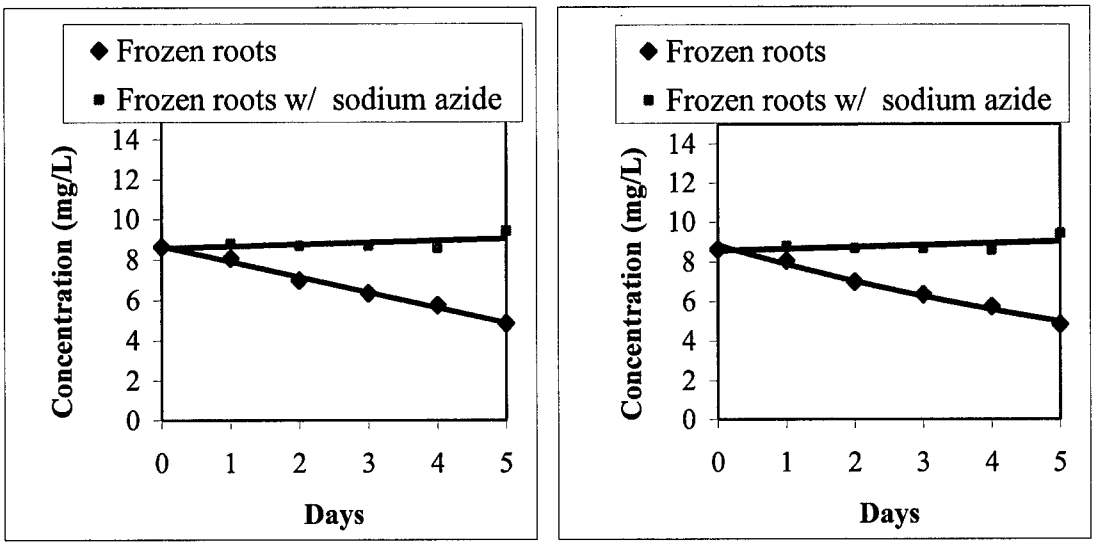

Figure 26: Linear and exponential curve fits for yellow nutsedge in hydroponic studies

Perchlorate was extracted from the roots and leaves of the grasses after completion of the hydroponic experiment. To determine the fate of perchlorate in the plant tissue, a mass balance was performed. Mass of perchlorate accumulated in the leaves and roots of big bluestem, Indian grass and yellow nutsedge are given in Table 6. Extremely high concentrations of perchlorate were accumulated in the leaves of Indian grass and yellow nutsedge and in the roots of Indian grass. The average concentration in the roots of Indian grass has a very high standard deviation, with actual values of 926.6 mg/kg and 71.4 mg/kg. This average was dominated by one analysis which maybe an aberration. The lower value of 71.4 mg/kg maybe more representative. Perchlorate was selectively translocated in the leaves with ratios of perchlorate concentrations found in the leaf to root of 15.5:1 in yellow nutsedge, 2.5:1 in big bluestem, and 0.4:1 in Indian grass. Accumulation of perchlorate in the leaves of yellow nutsedge was 732 ± 117 mg/kg, much higher than big bluestem and Indian grass.

Degradation products of perchlorate, chlorate, chlorite and chloride were found in both roots and leaves of all the grasses. Table 7, Table 8, and Table 9 show the amounts of chloride, chlorite and chlorate present in the grasses. Note that chloride is a natural ion, so its presence in the plant tissue may have nothing to do with perchlorate degradation. Yellow nutsedge was found to accumulate large amounts of chloride, chlorite and chlorate in the leaves. Significant amounts were also found in big bluestem, but were comparatively less than that found in yellow nutsedge. Indian grass appeared to accumulate a large amount of perchlorate degradation products but the standard deviation was significantly high in all the metabolites. Chlorite and chlorate appeared to have very high average concentration in the roots of live Indian grass, with replicate values of 6450 mg/kg and 850 mg/kg for chlorite and 3750 mg/kg and 280 mg/kg for chlorate. The average maybe dominated by one analysis which maybe an aberration. Hence, the lower value, 850 mg/kg and 280 mg/kg maybe more representative for chlorite and chlorate. Also, low perchlorate removal from the live plant of Indian grass (see Figure 22) indicates that the presence of degradation products should also be in low concentrations. Hence, it is more realistic to consider low concentrations for the degradation products of perchlorate in Indian grass. In almost all of the trials, the live plants accumulated more degradation products than the frozen controls. On average killed frozen roots accumulated fewer degradation products than the frozen roots, indicating that bacteria in the solution are degrading perchlorate.

Table 6: Mass of perchlorate accumulated in grasses in hydroponic studies

Plant	Concentration of perchlorate mg perchlorate/kg plant(dry)					
	Leaves		Roots		Frozen roots	Killed control
	Average	St. Dev	Average	St. Dev		
Yellow nutsedge	732	117	47.3	7.8	3.9	9.2
Indian grass	186	69.5	499	605	6.3	9.1
Big bluestem	102	49.2	40.4	3.9	10.3	10.01

Table 7: Mass of chloride in grasses in hydroponic studies

Plant	Concentration of chloride mg chloride/kg plant(dry)					
	Leaves		Roots		Frozen roots	Killed control
	Average	St. Dev	Average	St.Dev		
Big bluestem	1370	375	1023	5.5	159	43.4
Indian grass	84	119	59.2	42	76.9	0
Yellow nutsedge	7800	10350	1650	254	89.4	205

Table 8: Mass of chlorite in grasses in hydroponic studies

Plant	Concentration of chlorite mg chlorite/kg plant (dry)					
	Leaves		Roots		Frozen roots	Killed control
	Average	St. Dev	Average	St.Dev		
Big bluestem	879	90.6	1220	17.5	160	92
Indian grass	1080	253	3650	3960	22.2	34.3
Yellow nutsedge	11200	204	640	114	102	61.2

Table 9: Mass of chlorate in grasses in hydroponic studies

Plant	Concentration of chlorate Mg chlorate/kg plant (dry)					
	Leaves		Roots		Frozen roots	Killed control
	Average	St. Dev	Average	St.Dev		
Big bluestem	1600	353	496	18	192	67
Indian grass	417	148	2120	2310	183	32
Yellow nutsedge	3600	376	954	481	62.3	104

From Figure 27, 82% of the initially added perchlorate was found in the live plants of yellow nutsedge, 31% in big bluestem, and 25% in Indian grass. Accumulation of perchlorate in frozen roots of all of the grasses was less than 5%. In the killed control of all of the grasses, accumulation was less than 4% indicating that sorption of perchlorate to dead roots was insignificant.

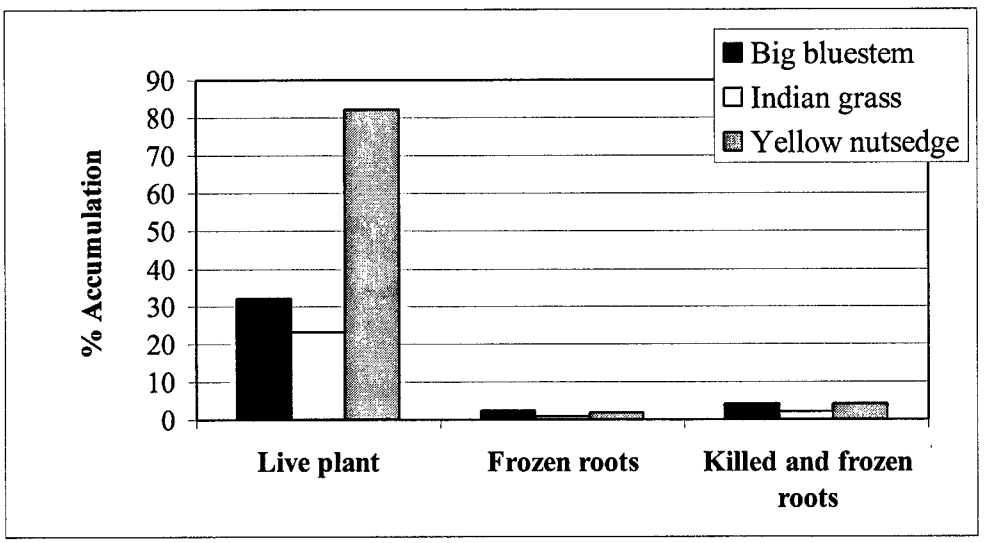

Figure 27: Uptake of perchlorate in hydroponic studies

From Table 10, it is evident that perchlorate degradation in the live plants of big bluestem and yellow nutsedge was at a slower rate when compared to Indian grass. Both yellow nutsedge and big bluestem were effective in selectively transpiring water and leaving perchlorate behind while Indian grass was not transpiring as much water but appeared to have degraded more perchlorate than yellow nutsedge and big bluestem. Degradation in the frozen roots of all the grasses was similar. Even though Indian grass demonstrated slow removal rates; it appeared to have degraded as much perchlorate as big bluestem and yellow nutsedge. There was significant degradation in the killed control of all the grasses but less than 5% of perchlorate accumulated in the dead roots. Hence,

sorption to roots was insignificant. In the frozen root controls of all the grasses, perchlorate degradation was higher than the live plant trials and killed control. This indicates an active microbial activity present in the roots of the plant initiating perchlorate degradation. Presence of breakdown products in the root and leaf tissue was due to active transformation of perchlorate by plant enzymes.

Table 10: Mass balance of perchlorate in hydroponic studies

Plant		% of Perchlorate				% degraded
		Leaves	Roots	Water	Total	
Big bluestem	live plant	20.6	10.9	30.7	62.2	37.8
	frozen roots killed	-----	2.3	29.2	31.5	68.5
	control	-----	3.9	60.1	64	36
Indian grass	live plant	14.0	9.7	10.8	34.5	65.5
	frozen roots killed	-----	0.8	30	30.8	69.2
	control	-----	1.9	74.9	76.8	23.2
Yellow nutsedge	live plant	72.1	6.6	0	78.6	21.4
	frozen roots killed	-----	1.7	25.9	27.6	72.4
	control	-----	4.1	63.9	68	32

The high percentage of perchlorate degraded in the frozen root controls indicates at the primary removal mechanism. Freezing kills the tissue and prevents production of new enzymes and the possibility that perchlorate was degraded by plant enzymes. Further, the killed control prevents bacterial activity and since there was significant difference in percent degradation between frozen root controls and killed controls, bacteria played an important role in perchlorate degradation. This explains why high percent degradation was observed in frozen root controls. In the live plant of Indian grass, the percent degraded was significantly higher than the other two grasses. This suggests that the plant had an active mechanism in perchlorate removal. Also, significant

degradation of perchlorate in the killed controls indicates that there was some passive mechanism involved in perchlorate degradation but was proceeding at extremely low rates.

Hence, bacteria played an important role in the degradation of perchlorate in the frozen root controls of all the grasses. The results of the live grasses were surprising as the live plants of all the grasses were degrading less perchlorate than the frozen root controls of the grasses. If there was bacterial degradation in frozen root controls, the same bacterial degradation should have also occurred in the live plant trials. The results of live Indian grass were intriguing. It appeared to degrade twice as much perchlorate as yellow nutsedge and big bluestem in the live plant. This indicates that high removal rates in the frozen root controls were possibly due to the dead roots stimulating bacterial activity by providing them nutrients and organics. Indian grass may have demonstrated more degradation by the presence of a larger bacterial community when compared to yellow nutsedge and big bluestem.

Perchlorate has been studied extensively for phytoremediation. In hydroponic systems, perchlorate degradation was found to be influenced by root-zone environments which affects the rate of degradation and determines the dominant mechanism (Nzengung et al., 2004). Rhizodegradation accounted for 96% of initial perchlorate removal in hydroponic reactors at the end of 40 days, with low dissolved oxygen and traces of nitrate. Low dissolved oxygen tends to slow the degradation rate to a large extent. Comparing these results with the results obtained in this study under normal conditions, approximately 70% of the initial perchlorate was rhizodegraded in the frozen root controls in 5 days which appears to be realistic. Two dominant mechanisms involved in

perchlorate removal by plants were phytodegradation of perchlorate and rhizodegradation (Nzengung et al., 1999). In this study, the significant decrease in perchlorate concentration in the live plant of Indian grass could possibly be due to phytodegradation where the plant along with microorganisms initiated perchlorate uptake and degradation. In Tan et al. perchlorate was also found to accumulate in the leaves and roots of plants. This was clearly observed in this study with yellow nutsedge, big bluestem and Indian grass. Smartweed hydroponic experiments indicate that the plant selectively transpired water leaving perchlorate behind in the solution (Tan et al., 2006). This behavior of smartweed was similar to that of the three grasses in this study. Large amounts of perchlorate were found in the leaves of smartweed which suggests that perchlorate uptake in plants generally accumulated in the leaves. Also, the accumulation of perchlorate in the tissue of the live plants was due to perchlorate uptake by transpiration via mass flow (Nzengung et al., 1999).

3.1.3. GTN

Concentrations of GTN in solutions decreased to zero within 40 hours from the start of experiments in big bluestem (Figure 28). An even faster decrease in concentration was seen in the frozen root control. In the dead root control, steady, but slower decreases in GTN concentration were evident. Nearly 100 % removal of GTN was seen in the live plants and the frozen root control of big bluestem. No degradation products were detected in any of the solutions. Significant loss was observed in the unplanted control with up to 38% removal in 39 hours. Still, this loss was significantly less than in the other treatments.

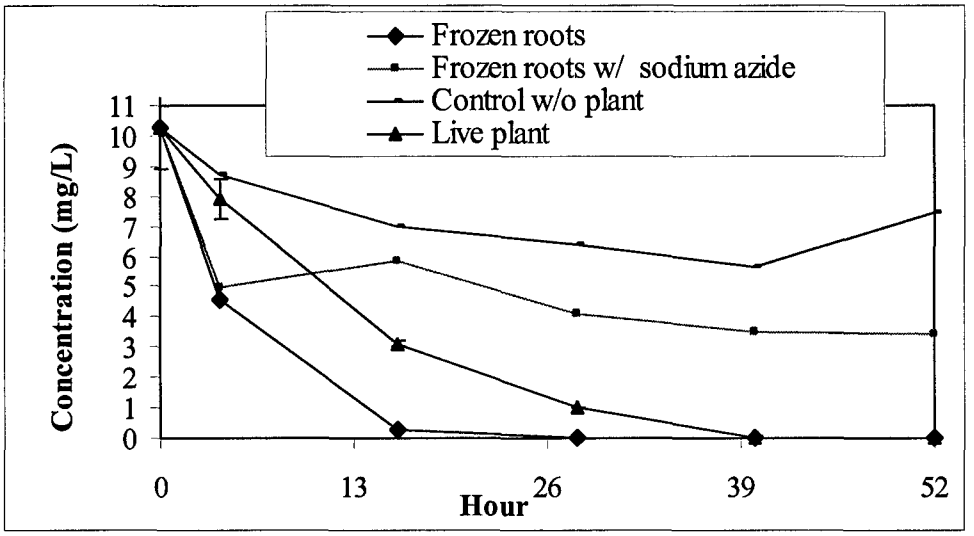

Figure 28: Concentration of GTN in big bluestem in hydroponic studies

Indian grass was also effective in degrading GTN as show in Figure 29. A rapid decrease in GTN concentrations were seen in both the live plants and the frozen root control. Decreases in GTN concentrations were at a slower rate in the dead root control. The live plant was seen to be effectively removing GTN from the solution. The concentration decreased from 10.3 mg/L to approximately 0 mg/L within 52 hours. No degradation products were detected in the solution. Significant loss was observed in the unplanted control, although the loss was less than in the other treatments.

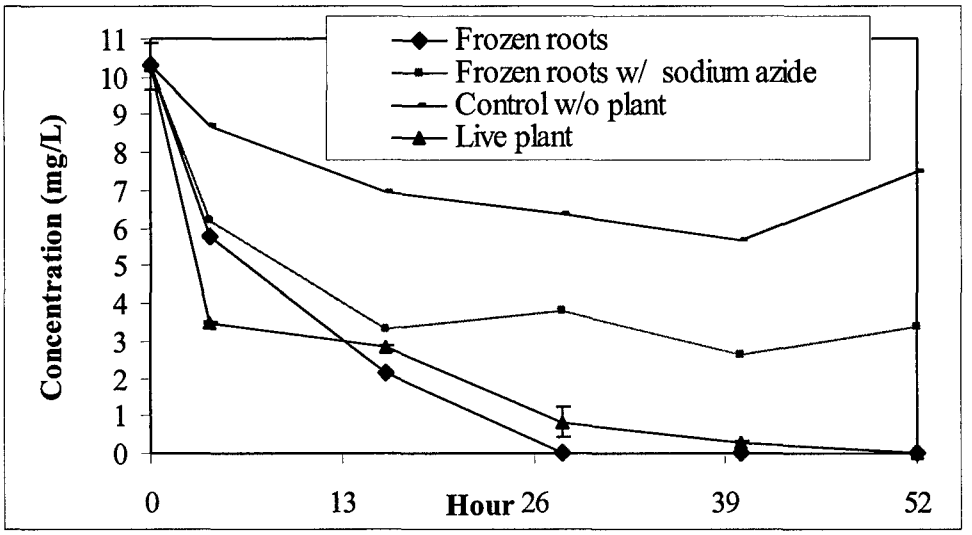

Figure 29: Concentration of GTN in Indian grass in hydroponic studies

Yellow nutsedge was found to transform GTN at a slower degradation rate than big blue stem and Indian grass. At the end of 52 hours, the concentration was reduced to 3 mg/L. This is consistent with the hydroponic results with 2,4-DNT that showed rapid removal with big bluestem and Indian grass, but slow incomplete removal with yellow nutsedge. Even though, the live plants were degrading GTN at a slower rate, the frozen root control achieved 100 % transformation at the end of 52 hours. The killed control showed slower removal rates approximately the same as the live plants. There was significant loss in the unplanted control, although less than in the other treatments.

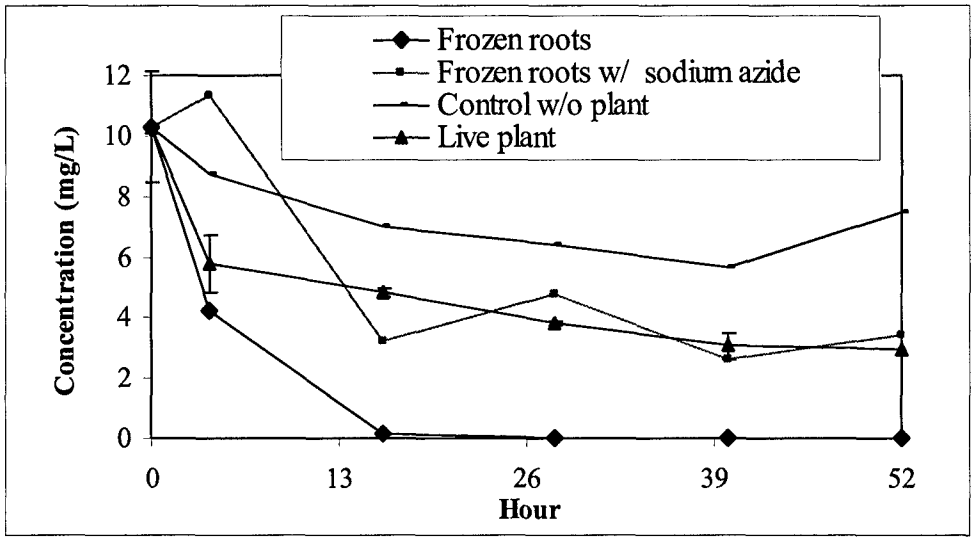

Figure 30: Concentration of GTN in yellow nutsedge in hydroponic studies

Rates of GTN removal are shown in Table 11, and curve fits are shown in Figure 31, Figure 32 and Figure 33. Linear and exponential curve fits were plotted for live plant, frozen root and killed control of the three grasses. The first order model has significantly better R^2 values that zero order model, indicating the first order model better depicts GTN removal. Live big bluestem and Indian grass had much higher GTN removal rates than live yellow nutsedge, however yellow nutsedge was found to have greater removal rates in the frozen root control when compared to big bluestem and Indian grass. Removal rates in the frozen root controls of big bluestem, Indian grass and yellow nutsedge exceed live plant removal rates and indicate significant bacterial activity. The killed control had significantly lower removal rates when compared to the frozen root control and live plant trials of all the grasses.

The four different treatments in this experiment were designed to isolate different mechanism of GTN removal. In the no plant control, hydrolysis reactions, sorption to glassware, and volatilization were measured. Significant losses by these mechanisms

were observed. GTN is the most readily degradable compound of the three tested in this study, so it is not surprising to have some loss in the control. The two frozen controls were frozen overnight to kill the plant tissue. These treatments measure passive sorption to roots in comparison to active uptake observed in the live plants. For all the grasses, removal from solution was rapid in the frozen root controls indicating GTN removal by either bacterial activity or due to sorption to roots. In the killed, frozen root control a limited amount of removal occurred indicating limited sorption and significant bacterial activity in the frozen root control. The removal rates in the frozen root controls were significantly higher when compared to the live plants of Indian grasses and yellow nutsedge. With big bluestem the frozen roots and live plants had similar removal rates. From this, it can be speculated that the dead roots were providing nutrients and organics for the bacterial community to thrive which could have initiated faster degradation of GTN when compared to the live grasses having limited microbial activity.

Table 11: Rate constants for GTN

Grass		Rate constant ($mg\ L^{-1}\ hr^{-1}$)	R^2	Rate constant (hr^{-1})	R^2
		Linear		Exponential	
Big bluestem	live plant	0.196	0.840	0.148	0.945
	frozen root	0.219	0.659	0.186	0.973
	killed control	0.097	0.587	0.017	0.703
Indian grass	live plant	0.152	0.653	0.101	0.941
	frozen root	0.237	0.806	0.220	0.906
	killed control	0.108	0.578	0.020	0.631
Yellow nutsedge	live plant	0.112	0.690	0.021	0.840
	frozen root	0.326	0.729	0.374	0.969
	killed control	0.153	0.656	0.024	0.647

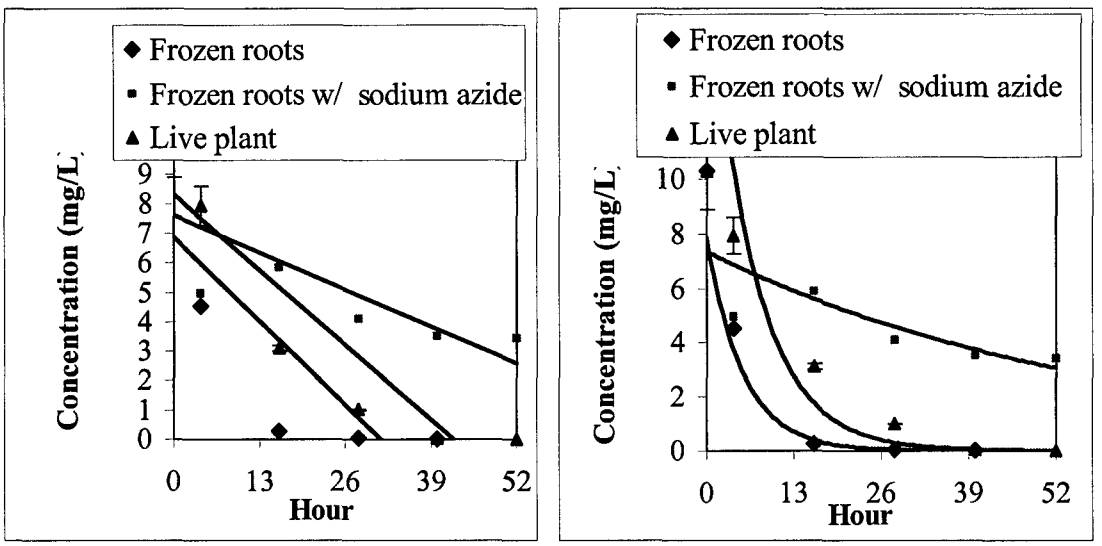

Figure 31: Linear and exponential curve fits for big bluestem in hydroponic studies

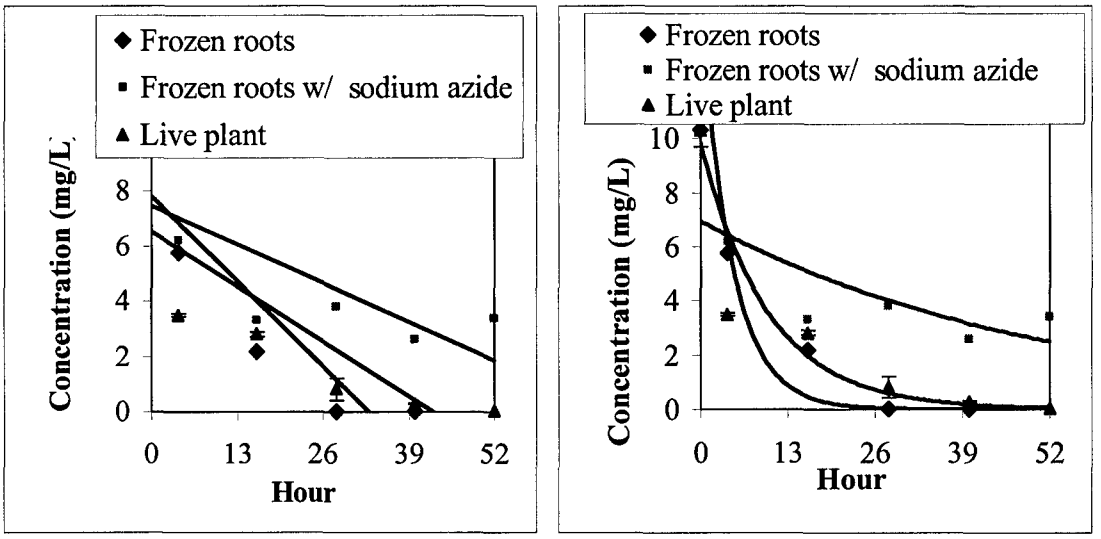

Figure 32: Linear and exponential curve fits for Indian grass in hydroponic studies

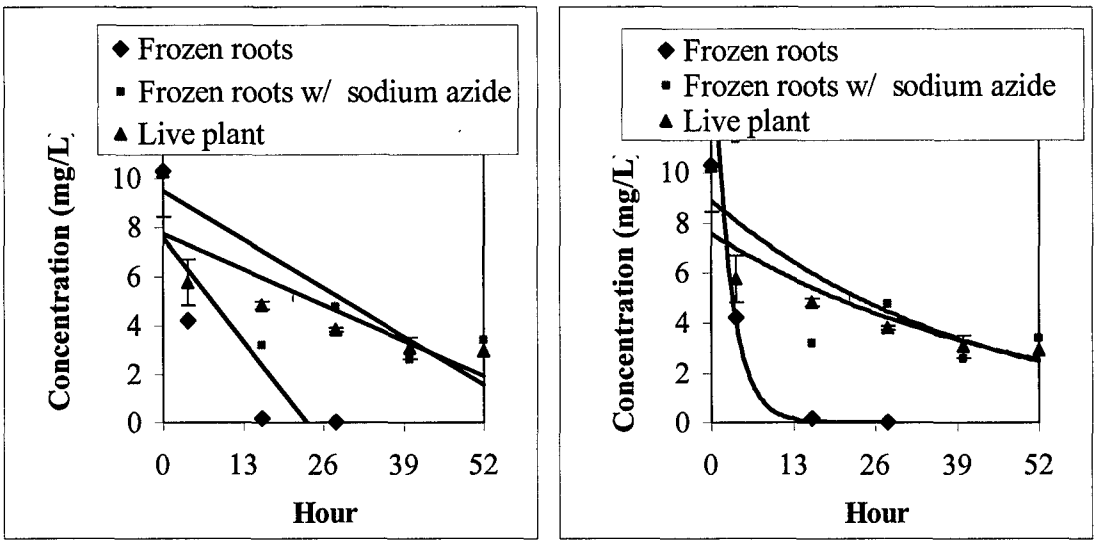

Figure 33: Linear and exponential curve fits for yellow nutsedge in hydroponic studies

After completion of the hydroponic experiments, plants were separated into leaves and roots, chopped, extracted, and analyzed by HPLC. Low levels of GTN accumulated in the leaves of live plants, big bluestem and Indian grass (Table 12). In Indian grass and big bluestem, roots of the live plants had high standard deviations. The replicate values were 0.2 mg/kg and 7.8 mg/kg for Indian grass and 6.7 mg/kg and 0.4 mg/kg for big bluestem. A more realistic value would be an average of both these values. Big bluestem saw uptake in frozen roots and killed control while no GTN concentrations were observed for Indian grass. In yellow nutsedge, no GTN accumulation was seen in the killed control. The presence of GTN in the leaf tissue indicates that GTN was actively taken up by the plants.

Table 12: Concentration of GTN accumulated in grasses in hydroponic studies

	Concentration of GTN mg GTN/kg plant (dry)					
	Leaves		Roots		Frozen roots	Killed control
	Average	St. Dev	Average	St.Dev		
Big bluestem	12.5	2.9	3.6	4.5	3.5	2.1
Indian grass	9.4	1.1	4.0	5.4	0	0
Yellow nutsedge	1.5	1.6	0.2	0.1	0.5	0

A mass balance was performed by comparing the initial mass added to the hydroponic solution with the mass detected in different media at the end of the experiment. Missing mass was assumed to have been degraded. Degradation of GTN was greatest in the frozen roots of the grasses, with 100% degradation in Indian grass. Live plants were also found to successfully degrade GTN with Indian grass being the most effective (97.6%). There was no significant accumulation of GTN in any of the plant. Sorption to dead roots was less than 0.7% in all grasses. Degradation in the unplanted control was the lowest of all totaling 27%.

The high percent of GTN degraded in the frozen roots indicate the primary removal mechanism. Freezing kills the tissue thereby preventing the production of new enzymes. Hence, GTN degradation was not degraded by plant enzymes. Further, the killed control prevents bacterial activity and since the rate of removal in the killed control was significantly less than in the frozen control, bacteria plays an important role in GTN degradation. Also, degradation of GTN in the live plant of yellow nutsedge was lower than Indian grass and big bluestem and also the frozen control of yellow nutsedge. Sorption of GTN to dead roots was low but significant; however bacterial degradation

was the dominant mechanism in GTN removal. Because of the low volatility of GTN, evapotranspiration was considered insignificant (Riefler and Medina, 2006).

Table 13: mass balance of GTN in hydroponic studies

Plant		% of GTN				% GTN degraded
		Leaves	Roots	Water	Total	
Big bluestem	live plant	3.8	0.6	0	4.4	95.6
	frozen roots	------	1.8	0	1.8	98.2
	killed control	------	1.5	56.5	58	42
Indian grass	live plant	1.6	0.8	0	2.4	97.6
	frozen roots	------	0	0	0	100
	killed control	------	0	20.1	20.1	79.9
Yellow nutsedge	live plant	0.8	0	9.2	9.7	80.3
	frozen roots	------	0.3	0	0.3	99.7
	killed control	------	0	26.5	26.5	73.5
Unplanted control		------	------	72.6	72.6	27.4

Phytoremediation of GTN contaminated soil has not been studied extensively in the past. GTN hydroponic experiments were performed in one study in order to test the ability of grasses to effectively treat contaminated soil (Riefler and Medina, 2006). In GTN hydroponic experiments, yellow nutsedge was able to remove 70% GTN in 3 d. This rate is consistent with this study where we saw 70% removal in 2 d (Figure 30). In Riefler and Medina, there was significant accumulation of GTN in the leaves and roots of yellow nutsedge, and in the killed controls. This is not consistent with the results of this study in which low concentrations were measured in roots and leaves.

3.2. Transplant Experiments

Yellow nutsedge, big bluestem and Indian grass were grown in potting soil for two months and then transplanted into beakers with live soil and sterile soil spiked with

2,4-DNT. Soil samples were collected every third day and analyzed for 2,4-DNT by HPLC. A 72% decrease in 2,4-DNT concentration occurred within 72 hours from the start of experiments in the live soil (Figure 34). This drop in concentration was not only seen in soil with live grasses but also in the unplanted control suggesting a removal mechanism unrelated to the plant. Note that the soil maintained a constant 2,4-DNT concentration for 30 days before the experiment, so that the 2,4-DNT loss appears to have been initiated by watering. Leaching however was not the cause, because there was no outlet at the bottom of the beaker and the total mass of 2,4-DNT extracted from the glass beads at the bottom of the beaker was less than 1%. It was suspected that bacterial activity reduced soil concentrations.

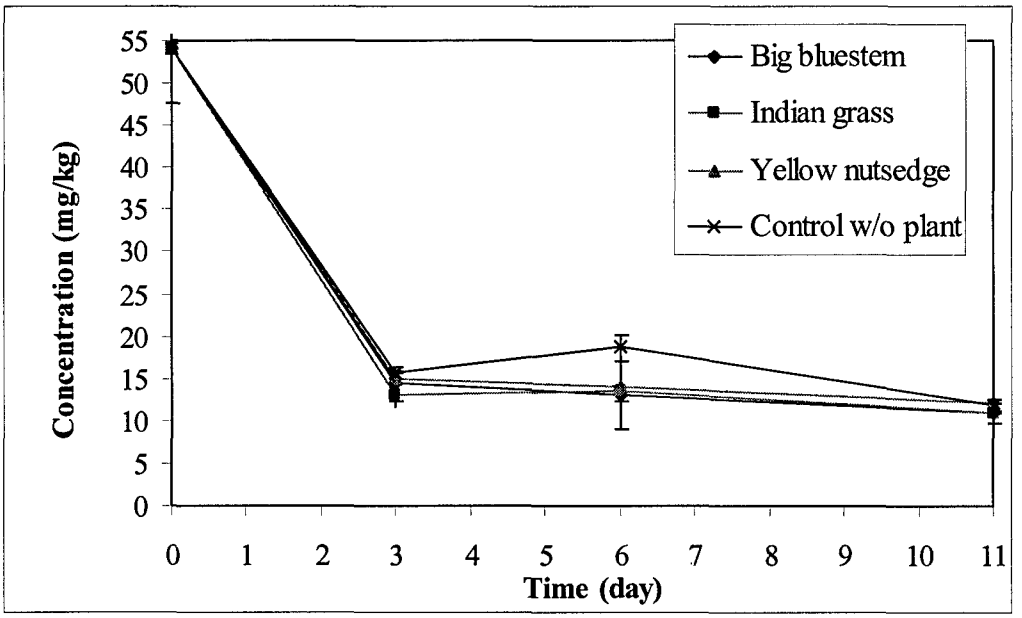

Figure 34: Concentration of 2,4-DNT in live soil

Figure 35 shows the concentration of 2,4-DNT in sterile soil. The drop in concentration in the sterile soil was similar to the unplanted soil, but the removal in

planted soil was slightly greater when compared to that of the unplanted soil. In any case, the plants had a minor effect on 2,4-DNT contamination over this short time frame.

There were multiple causes for the poor results in these experiments. First, the WES soil was extremely fine, and when the soil was transferred into beakers, it was compacted and did not provide adequate aeration for growth. The transplanted plants grew little in both live soil and sterile soil. The plants did not appear healthy but were surviving the contaminated conditions. Soil was made sterile by autoclaving it three times to kill spore forming microorganisms. But, bacterial contamination of the soil likely occurred while transplanting the plants from potting soil to beakers containing sterile WES soil. In addition, the sterile soil was watered with tap water and not sterile water and was open to the atmosphere. So, it is possible that the "sterile" soil was sufficiently contaminated for it to have similar results to the live soil. The alternative of cultivating strictly sterile plants was deemed too difficult. In addition problems often result from experiments using laboratory spiked soil. Soils briefly contaminated by a well distributed solvent are typically easier to treat than aged field contamination and complete removal in controls often results (Sung et al., 2003; Riefler and Medina, 2006). This was apparent in the 2,4-DNT experiment, and as a result, spiked soil studies were not completed with perchlorate and GTN. Instead, an alternative method to more realistically depict field contamination was developed called surface contamination experiments.

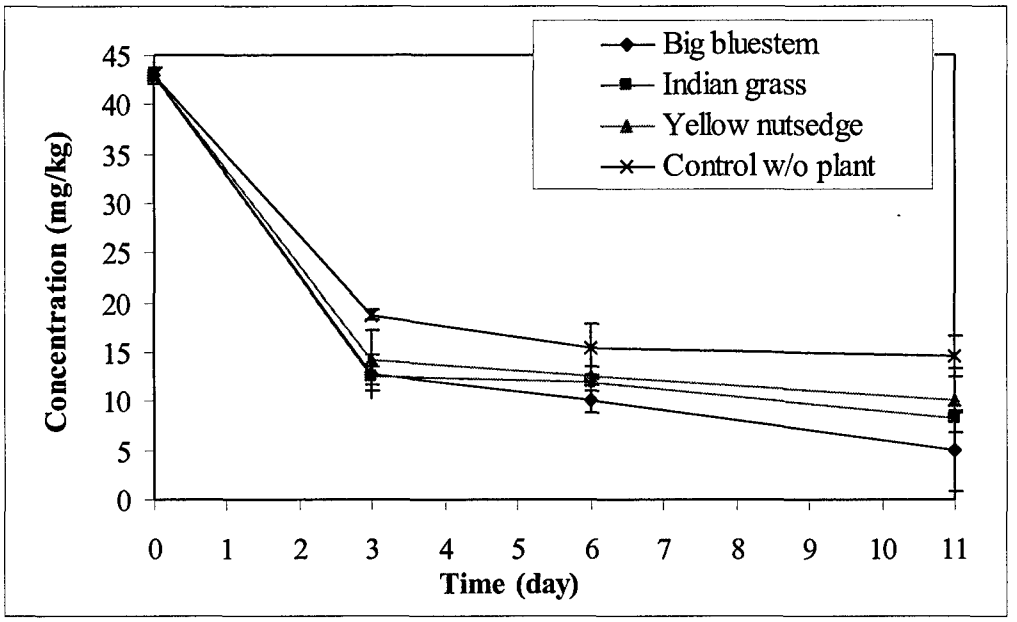

Figure 35: Concentration of 2,4-DNT in sterile soil

3.3. Germination experiments

WES soil was collected in beakers and planted with seeds of big bluestem, and Indian grass and corms of yellow nutsedge. The same was done in live soil and sterile soil both contaminated with 2,4-DNT. All the beakers were watered to field capacity. Yellow nutsedge germinated in the clean soil, live soil and sterile soil. Neither big bluestem nor Indian grass germinated from any of the beakers including the clean soil. This could be due to the poor quality WES soil compared to potting soil. WES soil was low in organic matter and uniformly fine grained. The compacted soil seemed to also inhibit germination. As yellow nutsedge had thicker roots and started from corms, the ability of the roots to penetrate the soil may have made growth possible compared to big bluestem and Indian grass which had small, fragile initial roots. Because of the difficulty encountered in working with spiked and sterile soil in the transplant experiments, these germination experiments were abandoned.

3.4. Surface contamination experiments

3.4.1. 2,4-DNT

The surface contamination experiments were designed to be more realistic with respect to contamination of firing ranges. Sources of contamination in firing ranges are mainly due to partially detonated ordnances leaching into the soil (Pennington et al., 2003). These contaminants pollute the soil and eventually seep through the ground, contaminating the water table. Even though this experiment was carried out on a lab scale, it was more realistic when compared to transplanted soil experiments.

Plants were grown two months prior to the start of experiments to ensure mature plant growth. 500 mg of pure 2,4-DNT crystals were spread over the soil of each container of yellow nutsedge and Indian grass. During daily watering, water was poured over the crystals to leach contamination into the soil. The ability of the plants to take up contamination from the soil and leachate was assessed. 2,4-DNT was extracted from the top portion of the soil (including the crystals), the bottom portion of soil, the glass beads, and from the leaf and root portions in each plant. Yellow nutsedge and Indian grass were chosen for these experiments because of their faster more vigorous growth than big bluestem.

Since 2,4-DNT has very low solubility, the crystals had not fully dissolved by the end of two weeks. This is evident from the high 2,4-DNT concentrations in the top soil (Table 14). The low concentration of 2,4-DNT in the top soil of controls may be due to the collected soil sample missing 2,4-DNT crystals. High concentrations in the bottom soil of unplanted control indicates the mobility of 2,4-DNT in the soil and that 2,4-DNT did not undergo degradation by bacteria in soil, evaporation, volatilization, sorption to

glassware, sorption to glass beads or reactions with soil. Large amounts of 2,4-DNT accumulated in the leaves and roots of Indian grass, while lower concentrations were seen in yellow nutsedge. The average concentrations of 2,4-DNT in the top layer of the soil and leaves of yellow nutsedge had high standard deviations. The top soil had replicate concentrations of 115 mg/kg and 3050 mg/kg while the leaves had 229 mg/kg and 43 mg/kg for their replicate concentrations. The low concentration in the top soil may be due to the collected sample missing crystals of 2,4-DNT and is not representative the actual. For the leaves of yellow nutsedge, an average of the two values may be realistic of 2,4-DNT. Low concentrations of transformation products, 2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene were found in leaves, roots and soil (Table 15 and Table 16). This demonstrates that degradation is taking place in the soil and plants. No degradation products were detected in the sterile control as expected.

Table 14: Concentrations of 2,4-DNT in surface contamination experiments

Beaker	Concentration of 2,4-DNT (mg/kg dry)							
	Top soil		Bottom soil		Leaves		Roots	
	Average	St.Dev	Average	St.Dev	Average	St.Dev	Average	St.Dev
Indian grass	924	64.8	12	7.5	1200	369	1600	96
Yellow nutsedge	1590	2100	62.5	0.1	136	132	67	0.8
Unplanted control	27.4	-----	484	-----	ND	-----	ND	-----
Sterilized control	54.3	-----	2.1	-----	ND	-----	ND	-----

Table 15: Mass of 2-amino-4-nitrotoluene in grasses in surface contamination experiments

Beaker	Mass of 2-amino-4-nitrotoluene (mg/kg dry)			
	Top soil		Bottom soil	
	Average	St.Dev	Average	St.Dev
Indian grass	2.88	2.03	0.79	----
Yellow nutsedge	ND	----	1.1	----
Unplanted control	ND	----	2.6	----

Table 16: Mass of 4-amino-2-nitrotoluene in grasses in surface contamination experiments

Beaker	Mass of 4-amino-2-nitrotoluene (mg/kg dry)							
	Top soil		Bottom soil		Leaves		roots	
	Average	St.Dev	Average	St.Dev	Average	St.Dev	Average	St.Dev
Indian grass	2.79	2.67	0.95	----	33.5	----	83.1	----
Yellow nutsedge	ND	----	1.55	0.38	ND	----	16.6	17.1
Unplanted control	ND	----	3.47	----	ND	----	ND	----

Percent 2,4-DNT degraded was obtained by performing a mass balance on 2,4-DNT present in the soil and plant (Table 17). Approximately 100% of 2,4-DNT was recovered from the unplanted live control, while significant loss of 2,4-DNT was seen in in live plants of yellow nutsedge and Indian grass and the unplanted sterilized control. An insignificant percentage of the 2,4-DNT was found sorbed to the glass drainage beads and the beakers.

Table 17: Mass Balance of 2,4-DNT in surface contamination experiment

Plant	% of 2,4-DNT					
	Leaves	Roots	Top soil	Bottom Soil	Beads	Total
Yellow nutsedge	0.746	0.475	6.5	1.28	0.056	9.06
Indian grass	0.011	0.046	13.4	0.385	0.041	13.88
Control	-----	-----	5.47	96.7	0.148	102.3
Sterilized	-----	-----	10.9	0.426	0.024	11.3

These totals are expressed as removal in Figure 36, which shows that 2,4-DNT was degraded effectively by yellow nutsedge and Indian grass with a mass removal of 92% in yellow nutsedge and 86% in Indian grass. In the unplanted control 0% was degraded, while in the sterilized control, 88.7% of initial 2,4-DNT was degraded. Maximum recovery in the unplanted control was found in the bottom soil which

illustrates the ability of 2,4-DNT to move through the soil. The solubility of 2,4-DNT is fairly high, 0.27 g/L of water at 22 ^{0}C (U.S.EPA, 1980). The results for the unplanted control and sterilized control are inconsistent. In the sterile soil, significant degradation of 2,4-DNT, 88.7% was observed, while no degradation was observed in the live soil. It is possible that autoclaving the soil resulted in a physical or chemical change to the soil enabling reactions with organic matter. These compounds are susceptible to abiotic humification i.e. covalent bonding of the chemical to organic macromolecules in soil (Achtnich et al., 2000). After this the chemical can no longer be extracted and detected.

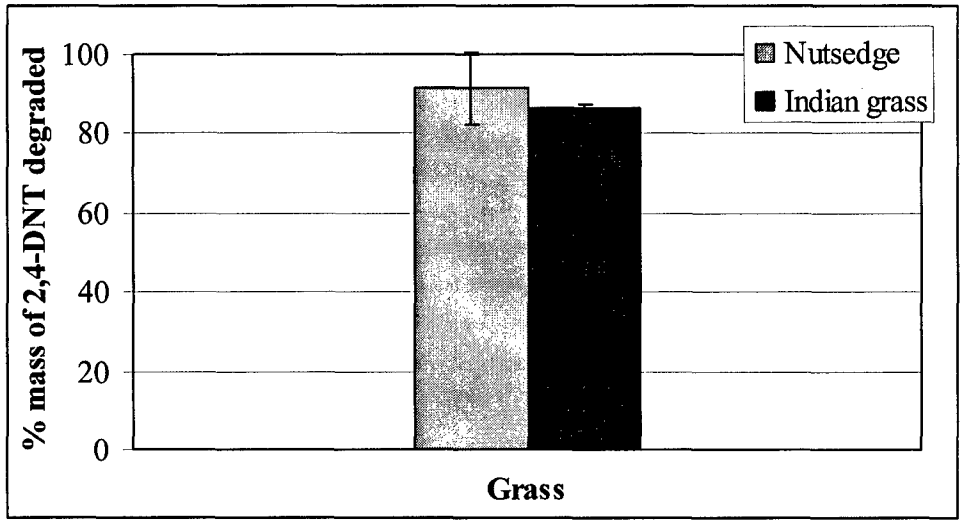

Figure 36: 2,4-DNT degradation in Yellow nutsedge and Indian grass

Nearly all of the 2,4-DNT was degraded in the soil of both yellow nutsedge and Indian grass. High concentrations of 2,4-DNT accumulated in Indian grass leaf and root, although it was a small portion of the total mass applied. This was consistent with the hydroponic experiments which also demonstrated 2,4-DNT accumulation. However, very high concentrations accumulated in Indian grass soil, different from the hydroponics on the yellow nutsedge growing on soil. 2,4-DNT was effectively degraded in the leaves,

roots, and soil which was evident from the presence of transformation products. As all the initial mass of 2,4-DNT was recovered from the unplanted live control, this study provides strong evidence that these plants prevented the leaching of 2,4-DNT into the soil and demonstrates that phytoremediation with grasses is likely a good treatment alternative for 2,4-DNT contaminated soil. Very high removal of 2,4-DNT in the sterile soil was inconsistent with the other control and may be a result of soil transformation by autoclaving of soil.

3.4.2. Perchlorate

616 mg of sodium perchlorate (500 mg ClO_4^-) were spread over the soil of yellow nutsedge and Indian grass. During daily watering, water was poured over the crystals to leach contamination into the soil. Perchlorate was extracted from the top portion of the soil, bottom portion of the soil, the glass beads, and from the roots and leaves of each plant. Since perchlorate is highly soluble in water, no perchlorate crystals were visible on the top layer of soil at the end of two weeks. Significant concentrations of perchlorate in the bottom portion of the soil indicate that perchlorate was mobile and has a high chance of being leached in the soil (Table 18). Perchlorate concentration in glass beads of the controls also indicates that perchlorate leached through the soil. In the unplanted control, loss of perchlorate in the soil could be due to bacteria present in the soil as perchlorate does not volatilize or evaporate, or sorb to the glassware. Loss of perchlorate in the sterile soil is difficult to explain. Large amounts of perchlorate accumulated in the leaves and roots of yellow nutsedge and Indian grass with higher accumulation in the leaves. This demonstrates the active uptake and accumulation of perchlorate from the soil pore water. The leaf to root ratio for yellow nutsedge was 5.5:1 and 7.5:1 in Indian grass. Chlorite

and chloride were also detected in the leaves and roots, and in the soil of yellow nutsedge and Indian grass indicating that perchlorate was degraded (Table 19 and Table 20). Note that chloride is a natural salt and it's presence in the plants may be unrelated to perchlorate degradation.

Table 18: Concentrations of perchlorate in surface contamination experiments

Beaker	Concentration of perchlorate (mg/kg dry)								
	Top soil		Bottom soil		Beads	Leaves		roots	
	Average	St.Dev	Average	St.Dev	mg	Average	St.Dev	Average	St.Dev
Indian grass	3950	3040	1100	285	0	47300	NA[a]	6290	NA[a]
Yellow nutsedge	2150	989	1159	305.7	0	46000	20500	8250	2710
unplanted control	12900	----	809	----	6.2	----	----	----	----
Sterilized control	2570	----	765	----	11	----	----	----	----

Note:
[a] As the biomass of leaves and roots for Indian grass was low, the leaves and roots of both the beakers were combined for a single analysis.

Table 19: Concentration of chlorite in surface contamination experiments

Beaker	Concentration of chlorite (mg/kg dry)								
	Top soil		Bottom soil		Beads	Leaves		roots	
	Average	St.Dev	Average	St.Dev	mg	Average	St.Dev	Average	St.Dev
Indian grass	13.1	8.1	9.8	5.1	0.8	0.3	----	1.1	----
Yellow nutsedge	5.2	3.8	4.8	1.6	0.9	12.7	12.2	2.7	1.6
Control	18.9	----	7.2	----	0.8	----	----	----	----
Sterilized	5.3	----	5.3	----	0	----	----	----	----

Table 20: Concentration of chloride in surface contamination experiments

Beaker	Concentration of chloride (mg/kg dry)								
	Top soil		Bottom soil		Beads	Leaves		roots	
	Average	St.Dev	Average	St.Dev	mg	Average	St.Dev	Average	St.Dev
Indian grass	5.6	2.3	3.5	2.1	0.2	0.2	----	0.2	----
Yellow nutsedge	1.6	0.9	1.2	0.1	0.2	5.5	6.7	1.0	0.5
Control	2.4	----	1.4	----	0.1	----	----	----	----
Sterilized	1.1	----	1.4	----	0	----	----	----	----

From the mass balance of perchlorate, it is evident that perchlorate degradation was significant not only in the yellow nutsedge and Indian grass, but also in the controls (Table 21). Even though Indian grass and yellow nutsedge had similar high concentrations in leaves and roots, the mass of perchlorate in Indian grass was much lower than yellow nutsedge, because of the much lower amount of biomass. Significant accumulation of perchlorate was seen in the leaves of yellow nutsedge. An insignificant percentage of the perchlorate was found sorbed to the glass drainage beads and the beakers.

Table 21: Mass balance of perchlorate in surface contamination experiments

Plant	% of Perchlorate					
	Leaves	Roots	Top soil	Bottom Soil	Beads	Total
Yellow nutsedge	17.4	2.5	12.9	5.79	0	38.6
Indian grass	0.8	0.8	23.7	6.6	0	31.9
Unplanted control	-----	-----	51.6	4.85	1	56.5
Sterilized control	-----	-----	10.3	4.59	1.9	14.9

61% degradation was seen in yellow nutsedge and 68% in Indian grass (Figure 37). In the controls used, 44% degradation was seen in the live soil and 85% degradation of perchlorate was seen in sterile soil. In the unplanted control, there was some active

mechanism rapidly degrading perchlorate. This is evident from low perchlorate concentrations in the soil and traces of metabolites.

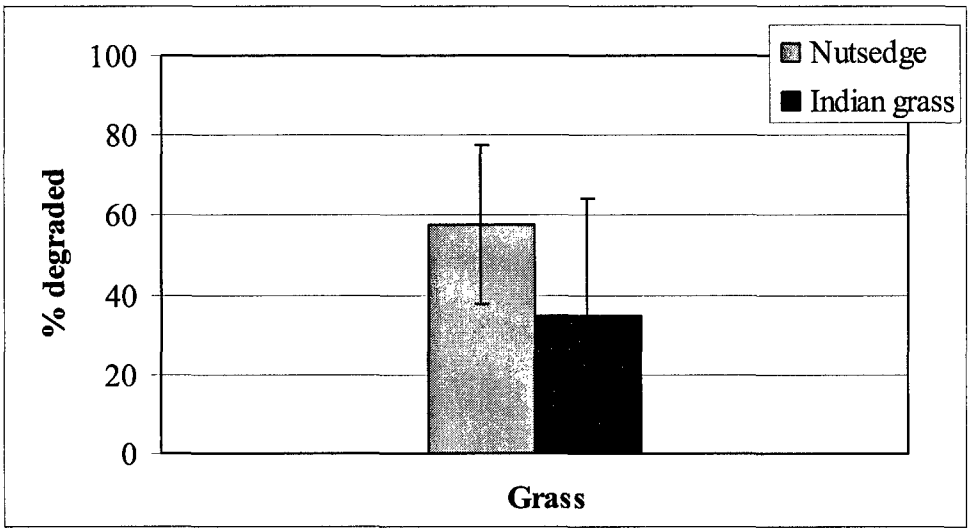

Figure 37: Degradation of perchlorate in yellow nutsedge and Indian grass

Hence, this study demonstrated very high perchlorate accumulation in the leaves and roots of the plants growing in contaminated soil with a clear preference for accumulation in the leaves. This is consistent with the hydroponic results, which also showed leaf and root accumulation, although at lower levels and with no clear preference. Plant enzymes and soil bacteria were likely transforming perchlorate which was evident from significant chlorite and chloride concentrations. A high percentage of perchlorate mass added to the soil was removed within two weeks in the planted treatments, however equally high mass removals were seen in the controls. These experiments show no net benefit to treating perchlorate contaminated soil by phytoremediation because of the rapid removal in unplanted soil. The plants were effective in taking up and accumulating perchlorate in their leaves, so these grasses were effective in phytoextraction.

3.4.3. GTN

Because of the explosion hazard, crystals were not used for the GTN experiments. Instead the plants were watered with GTN contaminated tap water (100mg/L). Yellow nutsedge and Indian grass were watered with GTN solution to field capacity. The amount of stock solution used in watering each plant was recorded. Since Indian grass was grown in 250 mL beakers compared to 25 mL crucibles used for yellow nutsedge, more stock solution was added to it. At the end of two weeks, uptake and transformation of GTN was determined by extracting it from the soil and plants. In the top portion of the soil, significant GTN concentrations were detected (Table 22). Low concentrations of GTN were seen in the bottom portion of the soil, with no GTN in the glass beads. Accumulation of GTN in the roots and leaves of both yellow nutsedge and Indian grass was low. More accumulation was seen in the leaf when compared to the root tissue in yellow nutsedge (2.5:1). Transformation products were not detected by the analytical method. GTN concentrations in the soil of the controls were low. In the top and bottom portions of the soil, concentrations in controls were similar to the yellow nutsedge results but much higher than the Indian grass results.

Table 22: GTN accumulation in roots and leaves in surface contamination experiments

Beaker	Mass of GTN (mg/kg dry)								
	Top soil		Bottom soil		Beads	Leaves		roots	
	Average	St.Dev	Average	St.Dev	mg	Average	St.Dev	Average	St.Dev
Indian grass	1.18	0.15	0.16	0.15	0.02	1.16	1.64	0	0
Yellow nutsedge	32.1	5.4	5.3	2.9	0.0004	5.04	1.9	1.57	2.22
Unplanted control	39	----	3	----	0.02	----	----	----	----
Sterilized control	29.8	----	9.4	----	0.025	----	----	----	----

Percent GTN recovered was calculated by a mass balance and is shown in Table 23. 94.6% removal of GTN was seen in yellow nutsedge, 99.4% removal in Indian grass, 92.7% in the unplanted live control and 90.3% in the sterilized unplanted control. On a mass basis extremely low accumulation of perchlorate was seen in the leaves and roots of the plants.

Table 23: Mass balance of GTN in surface contamination experiments

Plant	% of GTN							% Loss of GTN
	Leaves	Roots	Top soil	Bottom Soil	Beads	Leachate	Total	
Yellow nutsedge	0.052	0.031	1.762	0.822	0.003	2.760	5.429	94.6
Indian grass	0.001	0	0.408	0.041	0.147	-----	0.596	99.4
Control	-----	-----	6.243	0.889	0.169	-----	7.301	92.7
Sterilized	-----	-----	5.788	3.537	0.388	-----	9.714	90.3

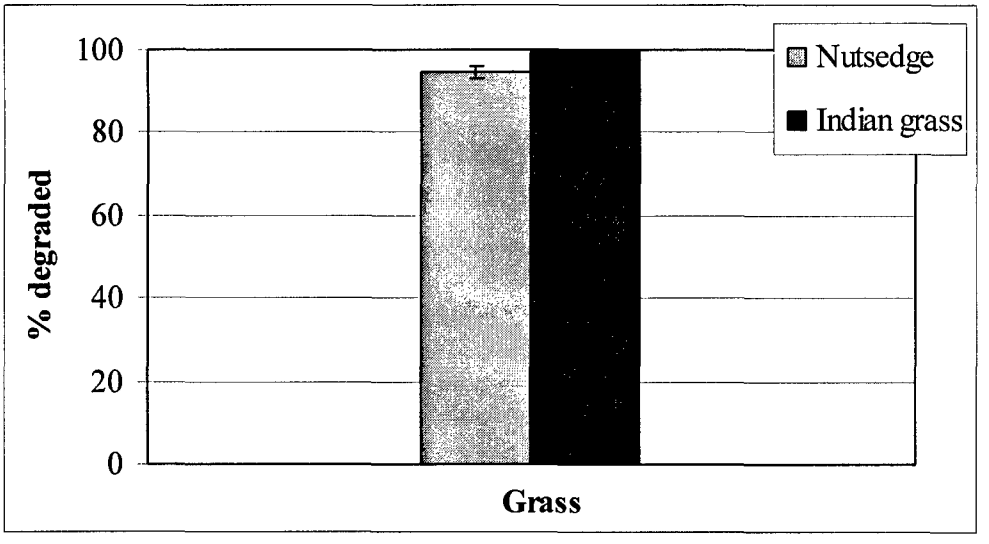

Figure 38: GTN degradation in yellow nutsedge and Indian grass

These results indicate that GTN was nearly completed degraded in all of the experiments including controls likely by bacteria in the soil. This is consistent with the hydroponic studies that showed the most rapid removal in solutions with frozen roots

dominated by bacterial degradation. In the case of low GTN concentration in sterile soil, contamination could have occurred during watering of the sterile control. Also, autoclaving of soil could have altered the physical/chemical properties of the soil enabling humification. Leachate from the yellow nutsedge plants was collected at the end of the experiment and analyzed for GTN by HPLC. GTN concentrations of 34 ± 9 mg/L were detected in the leachate, an average removal of 66%. These results indicate no apparent benefit to phytoremediation of soil contaminated with GTN by these grasses tested. It appears the soil's native biodegradation exceeds the plant driven removal mechanism.

4. Conclusions

In this study, the feasibility of grasses in cleaning up of propellant contaminated soil was observed by performing hydroponic and soil experiments. Three native grasses, yellow nutsedge, Indian grass and big bluestem, were used in clean-up of 2,4-DNT, perchlorate and GTN and their uptake and transformation were assessed.

Conclusions from these hydroponic and soil experiments include;

- Phytoremediation was likely effective for 2,4-DNT.
- 2,4-DNT was transformed in the roots and leaves of all three grasses, with maximum uptake in roots. Traces of breakdown products were found in extraction of the leaves of all plants indicating transformation in the plant by plant enzymes.
- Significant abiotic sorption of 2,4-DNT in the dead roots and minimal bacterial activity was seen in all plants indicating 2,4-DNT uptake is likely passive controlled by sorption.
- Yellow nutsedge appears to have a mechanism to minimize 2,4-DNT degradation because the percent degraded in live plants was significantly low compared to the frozen controls in hydroponic experiments.
- 2,4-DNT was not degraded in the soil by bacteria. This was evident from high 2,4-DNT concentration in the unplanted control in surface contamination experiments.
- Perchlorate was found to accumulate in the leaves and roots of the grasses.
- In perchlorate hydroponic studies, transformation products were found during extraction, but the degradation process on the whole was found to be taking place at a slower rate in the live plants. Bacterial transformation of perchlorate was evident in frozen root controls. But in the live plants of yellow nutsedge and big bluestem,

degradation of perchlorate was at a slower rate than the frozen root controls. Root zone conditions were found to be an influencing factor in perchlorate degradation (Nzengung et al. 2004). Also, organics and nutrients produced by dead roots could have provided the bacteria an environment to thrive on.

- GTN was rhizodegraded in the root zone of all plants in hydroponic and soil studies. Accumulation of GTN in roots and leaves were insignificant.
- Phytoremediation did not have any effect on GTN. In GTN and perchlorate, the unplanted controls failed to simulate field conditions where these contaminants persist. Sterilizing the soil by autoclaving altered the soil properties causing abiotic humification. Further, perchlorate is particularly mobile but was found in higher concentrations in the top portion of the soil than in the bottom portion of the soil in the controls. The controls had significant degradation of perchlorate and GTN indicating a possibility of soil biodegradation.
- Perchlorate and GNT had similar removal in live plants and controls. This indicates the failure to show effectiveness of phytoremediation. But, neither does it show its ineffectiveness. Phytoremediation could still be effective in field, only if a better experimental setup was designed to perform these studies.

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Appendix

Hydroponic experiments

i. 2,4-DNT

Table 24: Calibration data for 2,4-DNT and breakdown products

Standard	Concentration	Absorbance	Retention Time	Area	Calculated Conc.
	mg/L	au	min		mg/L
DNT					
	0.5	15771	13.15	207388.65	0.54
	2	56232	13.01	731578.32	1.90
	5	159836	12.85	2053892.60	5.34
	10	299716	12.75	3821379.00	9.94
4-amino-2-nitrotoluene					
	0.5	6937	6.37	44188.69	0.44
	2	24800	6.32	156736.00	1.57
	5	70838	6.24	442029.12	4.42
	10	131927	6.19	816628.13	8.17
2-amino-4-nitrotoluene					
	0.5	6794	6.85	46538.90	0.42
	2	32432	6.80	220537.60	1.98
	5	91439	6.72	614470.08	5.53
	10	170960	6.19	1058242.40	9.52

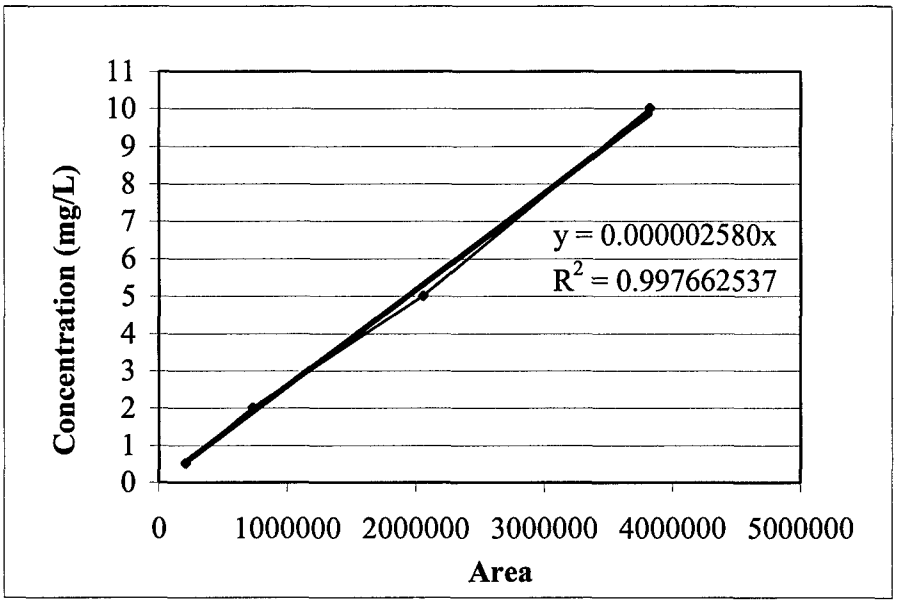

Figure 39: Calibration curve for 2,4-DNT

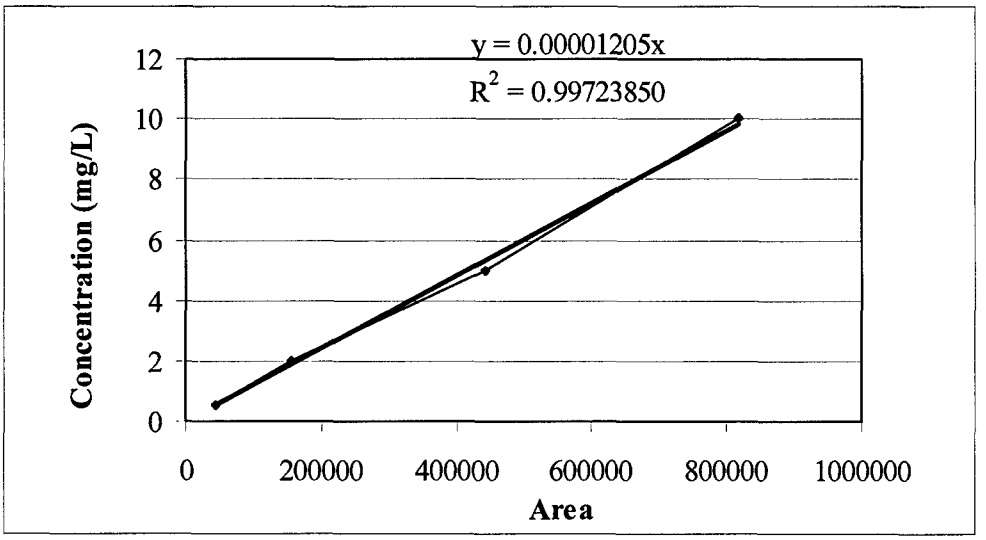

Figure 40: Calibration curve for 4-amino-2-nitrotoluene

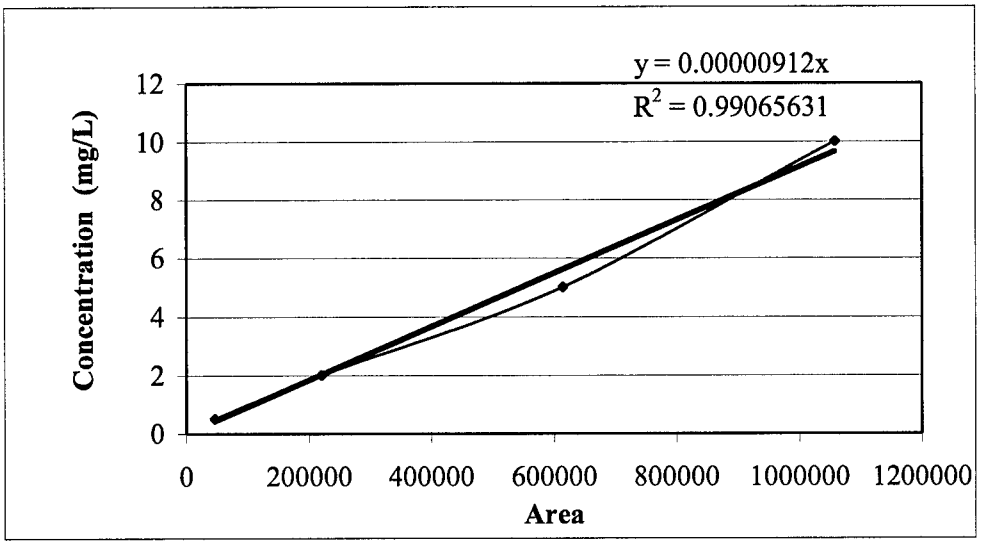

Figure 41: Calibration curve for 2-amino-4-nitrotoluene

Table 25:2,4-DNT concentration from hydroponic experiments

	Big bluestem				Indian grass				Yellow nutsedge				Unplanted control
			Frozen roots	Killed control			Frozen roots	Killed control			Frozen roots	Killed control	
# of Hours	conc.		conc.	conc.	conc.		conc.	conc.	conc.		conc.	conc.	conc.
Hr	mg/L		mg/L	mg/L	mg/L		mg/L	mg/L	mg/L		mg/L	mg/L	mg/L
	Average	Stdev			Average	Stdev			Average	Stdev			
0	7.70	0.23	7.70	7.70	7.70	0.23	7.70	7.70	7.70	0.23	7.70	7.70	7.70
12	2.14	1.89	4.22	3.87	3.70	0.71	4.41	4.52	6.01	0.41	5.25	5.48	7.61
24	1.13	1.33	0.93	0.67	0.16	0.18	2.73	3.35	5.66	0.53	3.32	3.67	6.83
36	0.40	0.05	0.62	0.66	0.17	0.02	0.68	0.26	4.64	0.39	2.16	1.74	7.05
48	0.44	0.08	0.79	0.65	0.20	0.01	0.55	0.51	4.89	0.52	2.02	0.57	6.65
60	0.68	0.09	0.95	0.99	0.29	0.06	0.83	0.76	5.33	1.03	0.62	0.50	7.19
72	0.52	0.15	0.52	0.55	0.27	0.05	0.37	0.40	4.61	0.38	0.93	0.37	5.88
84	0.57	0.06	0.35	0.32	0.28	0.14	0.34	0.25	3.59	0.83	0.90	0.49	5.71
96	0.59	0.07	0.23	0.23	0.43	0.07	0.30	0.10	0.06	0.08	0.65	0.40	4.97
108	0.47	0.07	0.14	0.18	0.42	0.10	0.17	0.12	2.41	0.38	0.62	0.29	4.02

Table 26: 2,4-DNT mass balance for hydroponic studies

	Initial Conc	Amount of 2,4-DNT added	Weight of wet leaves/roots taken	Moisture Content	Concentration of 2,4-DNT		Mass Balance %		
	mg/L	L	kg		mg/kg (dry)	mg/kg (wet)		average	stdev
yellow nutsedge -									
leaves #1	10	0.08	0.0015	0.76	23.83	5.62	3.41		
leaves # 2	10	0.11	0.0015	0.85	36.90	5.70	4.25	3.83	0.59
Roots	10	0.07	0.0015	0.93	107.68	7.75	22.37		
Frozen Roots	10	0.05	0.0015	0.92	106.49	8.40	27.76	31.60	
Killed control	10	0.05	0.0015	0.90	83.01	8.64	21.05		
leaves	10	0.06	0.0015	0.70	23.07	6.96	4.39		
Roots	10	0.06	0.0015	0.89	76.69	8.72	18.54	22.93	
Frozen Roots	10	0.05	0.0015	0.90	77.80	8.02	19.75		
Killed control	10	0.05	0.0015	0.90	84.52	8.45	21.13		
Big Blue Stem -									
leaves	10	0.09	0.0015	0.72	25.89	7.26	3.21		
Roots	10	0.09	0.0015	0.88	58.09	7.14	8.78	12.00	
Frozen Roots	10	0.05	0.0015	0.90	84.20	8.21	21.11		
Killed control	10	0.05	0.0015	0.88	70.17	8.33	17.50		

ii. Perchlorate

Table 27: Calibration data for perchlorate

Concentration	Retention Time	Area	Calculated conc.
mg/L	min	µs/min	mg/L
10	17.94	18.15	8.89
8.1	17.84	18.43	9.03
4.1	18.22	8.98	4.40
1.6	18.60	3.57	1.75
0.4	18.94	0.87	0.43

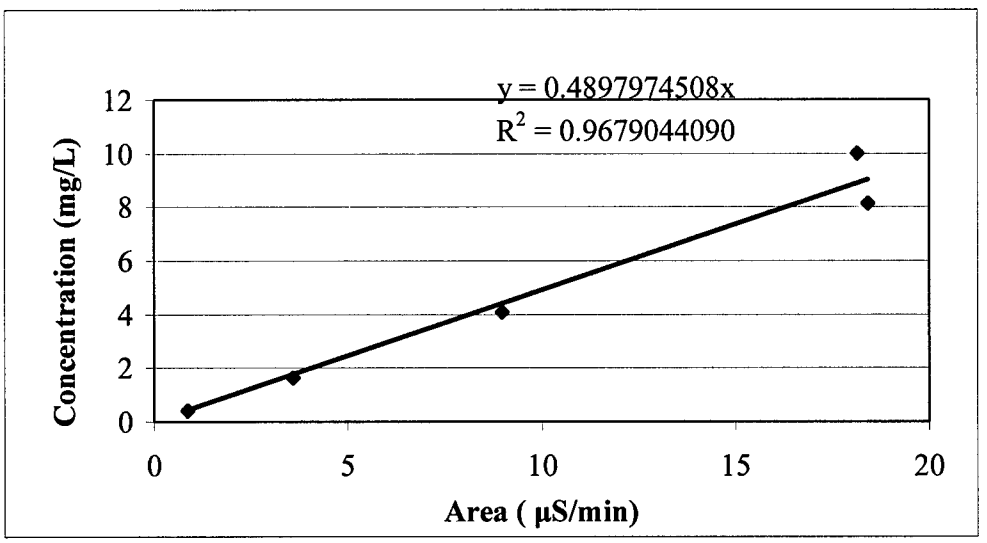

Figure 42: Perchlorate calibration curve

Table 28: Calibration data for chloride, chlorite and chlorate

Chlorite		
	Retention	
Concentration	Time	Area
mg/L	min	µs/min
7.46	3.623	46.4384
3.73	3.627	24.6047
1.49	3.6	12.4634
0.373	3.623	4.5265
Chloride		
	Retention	
Concentration	Time	Area
mg/L	min	µs/min
6.0687	3.917	58.1141
3.034	3.887	30.0051
1.2137	3.853	16.7245
0.3034	3.834	11.5515
Chlorate		
	Retention	
Concentration	Time	Area
mg/L	min	µs/min
7.84	4.683	59.4974
3.922	4.697	27.6561
1.569	4.5	2.2115
0.3922	4.513	0.1555

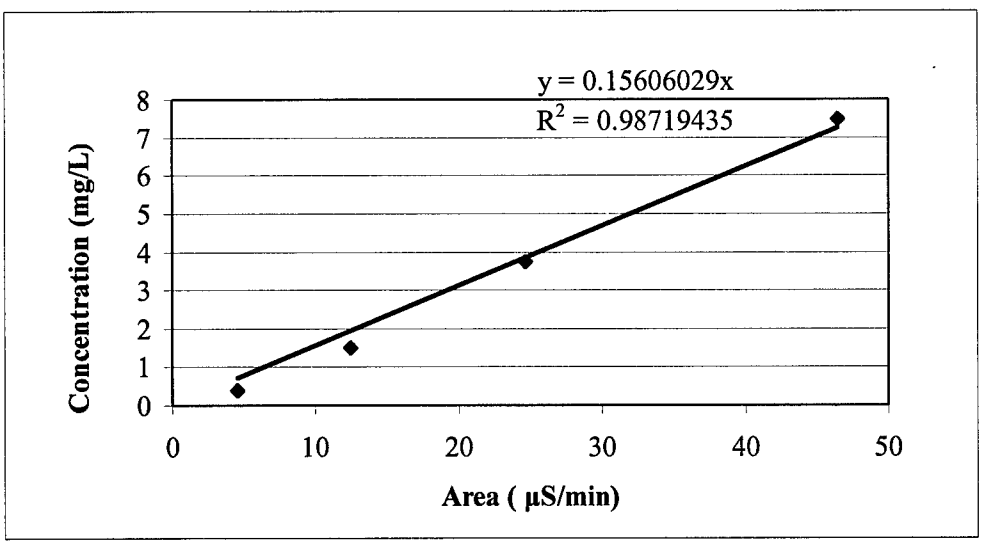

Figure 43: Calibration curve for chlorite

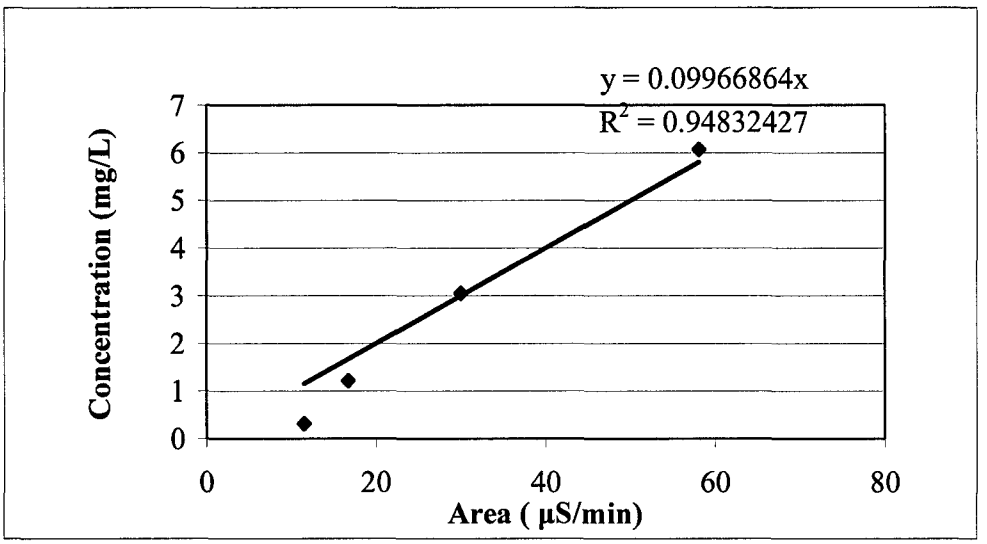

Figure 44: Calibration curve for chloride

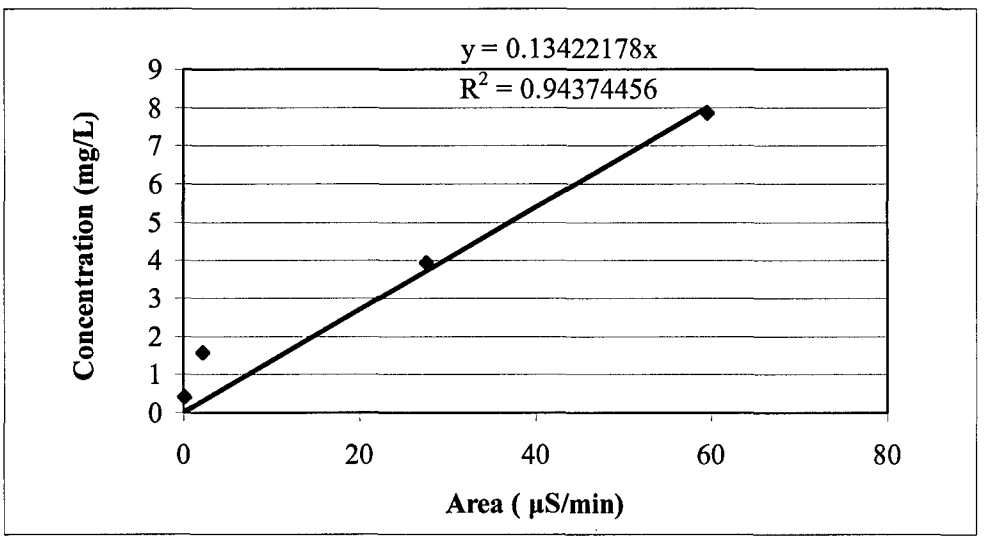

Figure 45: Calibration curve for chlorate

Table 29: Perchlorate concentration from hydroponic studies

	Big Blue Stem				Indian Grass				Yellow Nutsedge				Perchlorate Control
			Frozen Roots	Killed control			Frozen Roots	Killed control			Frozen Roots	Killed control	
# of Days	Conc.		Conc.	Conc.	Conc.		Conc.	Conc.	Conc.		Conc.	Conc.	Conc.
Day	mg/L		mg/L	mg/L	mg/L		mg/L	mg/L	mg/L		mg/L	mg/L	mg/L
	Average	Stdev			Average	Stdev			Average	Stdev			
0	8.61		8.61	8.61	8.61		8.61	8.61	8.61		8.61	8.61	8.61
1	8.99	0.16	6.96	8.44	8.73	0.03	8.50	8.65	9.62	0.20	8.05	8.78	8.89
2	10.12	0.20	6.57	8.35	9.49	0.04	9.17	9.52	10.18	0.07	6.97	8.67	9.29
3	11.36	0.67	6.19	8.41	9.58	0.10	8.70	9.85	10.73	0.32	6.32	8.67	8.97
4	12.68	0.43	5.39	8.36	10.08	0.13	8.71	9.72	11.42	0.45	5.72	8.57	9.09
5	16.05	0.85	4.97	8.02	10.86	0.34	8.39	8.27	13.62	0.37	4.82	9.41	9.11

Table 30: Mass balance of perchlorate in hydroponic experiments

	Initial Volume	Initial Conc.	Volume left			Conc. left	Volume transpired	Conc. transpired		Mass degraded	% degraded
Sample	V_{in}	C_{in}	$V_{L\ initial}$	V_{Water}	$V_{L\ final}$	C_L	V_T	C_T	$Vs.C_s$	M_{deg}	
#	mL	mg/L	mL			mg/L	mL	mg/L	mg	mg	
Big bluestem											
#1	123.3	8.61	5	5	10	27.291	83.3	0	0.30	0.79	7.88
#2	123.3	8.61	7.5	5	12.5	30.341	80.8	0	0.29	0.68	6.82
Frozen roots	103.3	8.61	69	5	74	3.515	0	0	0.15	0.63	6.29
Killed control	103.3	8.61	70	5	75	7.128	0	0	0.21	0.35	3.55
Indian grass #1	76.7	8.61	0	5	5	0.564	41.7	0	0.25	0.66	6.57
#2	76.7	8.61	7	5	12	11.691	34.7	0	0.24	0.52	5.20
Frozen roots	91	8.61	24.5	5	29.5	7.958	31.5	0	0.22	0.55	5.49
Killed control	91	8.61	56	5	61	9.620	35	0	0.23	0.20	1.96
Yellow nutsedge #1	156.7	8.61	0	5	5	0.029	121.7	0	0.27	1.35	13.48
#2	156.7	8.61	0	5	5	0.070	121.7	0	0.28	1.35	13.47
Frozen roots	103.3	8.61	66	5	71	3.247	2.3	0	0.16	0.66	6.59
Killed control	103.3	8.61	65.5	5	70.5	8.058	2.8	0	0.22	0.32	3.21

iii. GTN

Table 31: Calibration data for GTN

Concentration	Absorbance	Retention Time	Area	Calculated Conc
mg/L	au	min		mg/L
0.5	9700	7.23	70131	0.58
2	19250	7.25	139559	1.15
5	54354	7.25	394067	3.24
10	178665	7.23	1291748	10.62

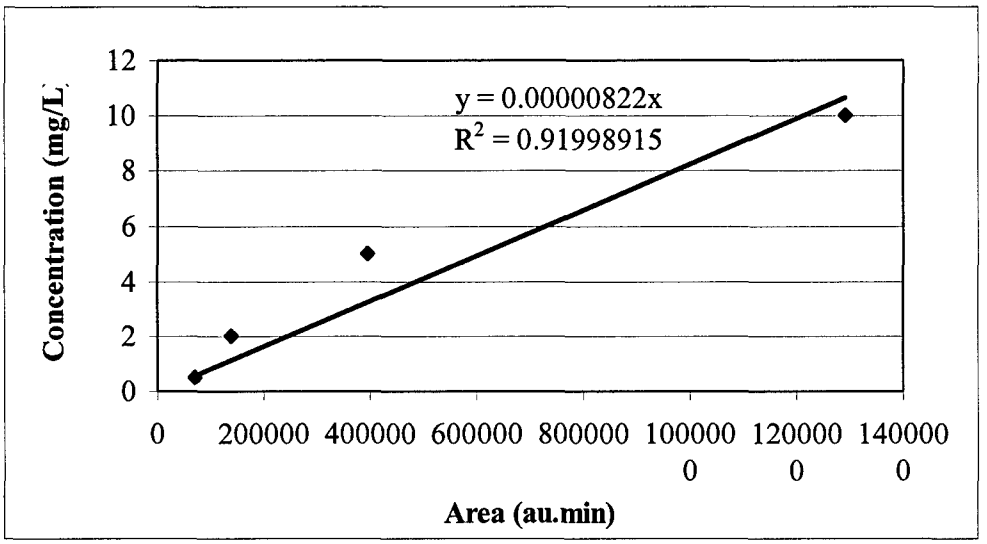

Figure 46: Calibration curve for GTN

Table 32: GTN concentrations from hydroponic experiments

	Big Blue Stem				Indian Grass				Yellow Nutsedge				Nitroglycerin Control
			Frozen roots	Killed control			Frozen Roots	Killed control			Frozen Roots	Killed control	
#	Conc.		Conc.	Conc.	Conc.		Conc.	Conc.	Conc.		Conc.	Conc.	Conc.
Hour	mg/L		mg/L	mg/L	mg/L		mg/L	mg/L	mg/L		mg/L	mg/L	mg/L
	Average	Stdev			Average	Stdev			Average	Stdev			
0	10.29		10.29	10.29	10.29		10.29	10.29	10.29		10.29	10.29	10.29
4	7.93	1.39	4.53	4.94	3.49	0.62	5.74	6.18	5.76	1.83	4.20	11.32	8.71
16	3.12	0.66	0.26	5.86	2.81	0.04	2.16	3.30	4.83	0.94	0.14	3.20	6.96
28	1.01	0.10	0.02	4.09	0.82	0.09	0.01	3.80	3.83	0.16	0.00	4.75	6.34
40	0.03	0.01	0.01	3.51	0.29	0.40	0.00	2.60	3.06	0.08	0.00	2.58	5.65
52	0.01	0.01	0.00	3.42	0.03	0.01	0.01	3.39	2.93	0.44	0.03	3.39	7.47

Table 33: Mass balance of GTN in hydroponic experiments

	Initial Volume	Initial Conc.	Volume left			Conc. left		Mass degraded	% degraded
Sample	V_{in}	C_{in}	$V_{L\ initial}$	V_{Water}	$V_{L\ final}$	C_L	$V_s.C_s$	M_{deg}	
#	mL	mg/L	mL			mg/L	mg	mg	
Big bluestem #1	35.6	10.3	0	5	5	0.01	0.01	0.37	3.7
#2	35.6	10.3	0	5	5	0.03	0.01	0.37	3.7
Frozen roots	37.6	10.3	29	0	29	0.00	0.00	0.39	3.9
Killed control	81.6	10.3	79	0	79	6.00	0.02	0.36	3.6
Indian grass #1	31.0	10.3	4.25	0	4.25	0.02	0.01	0.32	3.2
#2	44.0	10.3	10.5	0	10.5	0.02	0.01	0.45	4.5
Frozen roots	15.0	10.3	11.5	0	11.5	0.00	0.01	0.15	1.5
Killed control	11.0	10.3	9.5	0	9.5	2.39	0.01	0.09	0.9
Yellow nutsedge									
#1	64.3	10.3	26.5	0	26.5	2.70	0.02	0.59	5.9
#2	52.3	10.3	24	0	24	1.68	0.01	0.50	5.0
Frozen roots	43.0	10.3	53.25	0	53.25	0.00	0.00	0.44	4.4
Killed control	52.3	10.3	50	0	50	2.85	0.02	0.40	4.0

Transplanted experiments

Table 34: 2,4-DNT concentration in live soil in transplanted experiments

	Big bluestem		Indian grass		Yellow nutsedge		Control	
Time day	Conc. mg DNT/kg dry soil	Std dev.	Conc. mg DNT/kg dry soil	Std dev.	Conc. mg DNT/kg dry soil	Std dev.	Conc. mg DNT/kg dry soil	Std dev.
0	53.95	6.33	53.95	6.33	53.95	6.33	53.95	6.33
3	14.44	2.00	13.04	1.37	14.99	3.02	15.74	0.15
6	13.02	3.96	13.60	1.09	14.02	0.91	18.64	1.58
11	10.96	1.16	10.93	0.51	11.98	0.72	11.75	0.90

Table 35: 2,4-DNT concentration in sterile soil in transplanted experiments

	Big bluestem		Indian grass		Yellow nutsedge		Control	
Time day	Conc. mg DNT/kg dry soil	std dev.	Conc. mg DNT/kg dry soil	std dev.	Conc. mg DNT/kg dry soil	Std dev.	Conc. mg DNT/kg dry soil	Std dev.
0	42.91	0.80	42.91	0.80	42.91	0.80	42.91	0.80
3	12.64	0.85	12.51	2.34	14.11	3.11	18.76	0.47
6	9.98	1.15	11.83	0.46	12.56	0.93	15.38	2.59
11	4.88	4.11	8.32	0.52	10.08	3.37	14.56	2.10

Surface contamination experiments

i. 2,4-DNT

Table 36: 2,4-DNT concentration from extraction in surface contamination experiments

Beaker	Retention Time	Absorbance	Concentration	Amount of Acetonitrile added	Amount of Soil/leaf/root added	Moisture Content	Wet weight	Dry Weight	Dry Mass	Mass of 2,4 - DNT
#	min	au	mg/L	L	kg		mg/kg	mg/kg	kg	mg
Yellow nutsedge										
#1										
Top soil	9.89	9930804	253.39	0.02	0.03	0.03	111.79	115.53	0.03	3.93
bottom soil	9.44	6060988	147.61	0.02	0.04	0.14	53.48	62.42	0.10	6.22
beads	9.47	120184	2.94	0.02						0.04
leaves	9.41	860743	20.90	0.02	0.00	0.02	223.89	229.02	0.00	0.32
roots	9.44	401271	9.77	0.02	0.00	0.20	54.29	67.59	0.00	0.18
#2										
Top soil	9.65	40454003	4028.66	0.02	0.02	0.00	3036.67	3050.30	0.02	60.70
bottom soil	9.39	8323937	201.65	0.02	0.05	0.00	62.24	62.52	0.11	6.61
beads	9.36	1443208	34.85	0.02				0.00		0.52
leaves	9.52	361377	8.88	0.02	0.00	0.08	39.16	42.57	0.08	3.41
roots	9.49	1027631	25.16	0.02	0.01	0.07	61.87	66.44	0.07	4.57
Indian grass										
#1										
Top soil	9.60	25704238	2546.52	0.02	0.05	0.16	816.19	969.31	0.06	63.00
bottom soil	9.44	2243268	54.63	0.02	0.05	0.13	15.09	17.33	0.13	2.23
beads	9.55	159522	3.93	0.02				0.00		0.06
leaves	9.49	53485	1.31	0.02	0.00	0.00	1455.01	1457.04	0.00	0.02
roots	9.31	442888	10.64	0.02	0.00	0.00	1651.84	1655.95	0.00	0.16
#2										
Top soil	9.60	40071600	3969.90	0.02	0.08	0.16	734.26	877.64	0.08	71.18
bottom soil	9.57	1386680	34.24	0.02	0.09	0.12	5.86	6.67	0.24	1.62
beads	9.47	953563	23.30	0.02				0.00		0.35
leaves	9.28	260057	6.23	0.02	0.00	0.00	933.94	935.24	0.00	0.09
roots	9.17	851525	20.15	0.02	0.00	0.01	1510.91	1520.67	0.00	0.30
Control										
Top soil	9.31	40533798	973.59	0.02	0.03	0.09	577.23	633.35	0.04	27.36
bottom soil	9.36	45639404	1102.12	0.02	0.03	0.13	635.84	729.38	0.66	483.58
beads	9.31	2040284	49.01	0.02						0.74
Sterilized										
Top soil	9.68	35569200	3553.21	0.02	0.04	0.02	1417.50	1445.22	0.04	54.34
bottom soil	9.36	1623809	39.21	0.02	0.03	0.13	22.20	25.39	0.08	2.13
beads	9.44	341711	8.32	0.02						0.12

Table 37: Mass balance of 2,4-DNT in surface contamination experiments

Beaker #	Min mg	Conc. mg	Mass degraded mg	% mass degraded
Yellow nutsedge				
# 1				
Top soil		3.93		
bottom soil		6.22		
beads		0.04		
leaves		0.32		
roots	500	0.18	489.31	98
# 2				
Top soil		60.70		
bottom soil		6.61		
beads		0.52		
leaves		3.41		
roots	500	4.57	424.18	85
Indian grass				
# 1				
Top soil		63.00		
bottom soil		2.23		
beads		0.06		
leaves		0.02		
roots	500	0.16	434.53	87
# 2				
Top soil		71.18		
bottom soil		1.62		
beads		0.35		
leaves		0.09		
roots	500	0.30	426.45	85
Control				
Top soil		27.36		
bottom soil		483.58		
beads	500	0.74	-11.68	-2
Sterilized				
Top soil		54.34		
bottom soil		2.13		
beads	500	0.12	443.40	89

ii. Perchlorate

Table 38: Perchlorate concentration in surface contamination experiments

Beaker #	Retention Time min	Area au	Conc. mg/L	Amount of NaOH added L	Amount of Soil/leaf/root added kg	Moisture Content	Wet weight mg/kg	Dry Weight mg/kg	Dry Mass kg	Mass of Perchlorate mg
Yellow nutsedge										
# 1										
Top soil	8.18	2973.90	7283.05	0.02	0.03	0.01	2835.74	2852.86	0.03	74.17
bottom soil	8.30	3271.01	1602.13	0.02	0.02	0.03	1335.11	1375.25	0.06	84.72
beads	11.74	871.85	427.03	0.02						8.54
leaves	9.91	1505.30	3686.47	0.04	0.00	0.06	56714.87	60457.53	0.00	157.19
roots	11.17	1179.10	577.52	0.04	0.00	0.01	10043.79	10169.86	0.00	23.39
# 2										
Top soil	7.92	3642.57	1784.12	0.02	0.03	0.15	1234.69	1453.59	0.03	42.01
bottom soil	8.89	2484.57	1216.94	0.02	0.03	0.17	785.12	942.94	0.06	56.39
beads	10.82	1174.70	575.36	0.02						11.51
leaves	9.94	1863.80	912.88	0.04	0.00	0.04	30177.92	31497.87	0.00	56.70
roots	13.79	386.82	189.46	0.04	0.00	0.01	6263.20	6331.20	0.00	7.66
Indian grass										
# 1										
Top soil	8.17	3040.69	7446.61	0.02	0.03	0.11	5415.71	6099.24	0.03	167.73
bottom soil	7.88	3537.80	1732.80	0.02	0.03	0.10	1166.87	1300.03	0.07	86.58
beads	11.23	927.08	454.08	0.02						9.08
# 2										
Top soil	7.53	4091.10	2003.81	0.02	0.03	0.17	1489.82	1795.02	0.17	305.20
bottom soil	8.79	2383.61	1167.49	0.02	0.03	0.17	746.00	897.71	0.23	207.46
beads	10.96	1013.20	496.26	0.02						9.93
leaves	14.67	241.37	118.22	0.04	0.00	0.00	47289.63	47289.63	0.00	4.73
roots	14.57	255.95	125.36	0.04	0.00	0.00	6268.14	6288.98	0.00	5.03
Control										
Top soil	8.95	2200.67	10778.80	0.02	0.02	0.01	12755.98	12939.64	0.02	218.68
bottom soil	8.93	2387.10	1169.20	0.02	0.03	0.09	733.04	809.17	0.12	93.62
beads	12.21	631.55	309.33	0.02						6.19
Sterilized										
Top soil	10.37	1286.00	3149.39	0.02	0.02	0.00	2560.48	2572.59	0.02	63.29
bottom soil	9.51	1824.14	893.46	0.02	0.03	0.12	674.31	765.06	0.10	79.95
beads	11.17	1168.30	572.23	0.02						11.44

Table 39: Mass balance of perchlorate in surface contamination experiments

Beaker #	M_{in} mg	Conc. mg	Mass degraded mg	% mass degraded
Yellow nutsedge				
# 1				
Top soil		74.17		
bottom soil		84.72		
beads		8.54		
leaves		157.19		
roots	615.57	23.39	**267.56**	**43**
# 2				
Top soil		42.01		
bottom soil		56.39		
beads		11.51		
leaves		56.70		
roots	615.57	7.66	**441.31**	**72**
Indian grass				
# 1				
Top soil		167.73		
bottom soil		86.58		
beads	615.57	9.08	**342.42**	**56**
# 2				
Top soil		305.20		
bottom soil		207.46		
beads	615.57	9.93	**83.23**	**14**
leaves		4.73		
roots		5.03		
Control				
Top soil		218.68		
bottom soil		93.62		
beads	615.57	6.19	**297.08**	**52**
Sterilized				
Top soil		63.29		
bottom soil		79.95		
beads	615.57	11.44	**460.89**	**75**

iii. GTN

Table 40: GTN concentration in surface contamination experiments

Beaker	Retention Time	Absorbance	Concentration	Amount of Acetonitrile added	Amount of Soil/leaf/root added	Moisture Content	Wet weight	Dry Weight	Dry Mass	Mass of GTN
#	min	au	mg/L	L	kg		mg/kg	mg/kg	kg	mg
Yellow nutsedge										
#1										
Top soil	7.95	145397	9.50	0.01	0.0034	0.01	27.95	28.24	0.00	0.10
bottom soil	7.97	56660	3.71	0.01	0.0122	0.06	3.04	3.22	0.01	0.04
beads	0	0	0.00	0.01						0.00
leaves	7.97	4793	0.31	0.01	0.0005	0.02	6.28	6.38	0.00	0.00
roots	7.92	6648	0.43	0.01	0.0014	0.02	3.09	3.14	0.00	0.00
#2										
Top soil	7.81	226238	14.52	0.01	0.0042	0.04	34.58	35.86	0.00	0.15
bottom soil	7.87	102676	6.64	0.01	0.0103	0.12	6.45	7.35	0.01	0.08
beads	8	645	0.04	0.01						0.00
leaves	7.84	6044	0.39	0.01	0.0011	0.04	3.54	3.70	0.00	0.00
roots	0	0	0.00	0.01	0.0014	0.05	0.00	0.00	0.00	0.00
Indian Grass										
#1										
Top soil	7.95	42372	2.77	0.01	0.0143	0.08	1.94	2.10	0.01	0.11
bottom soil	8	13208	0.87	0.01	0.0367	0.13	0.24	0.27	0.04	0.01
beads	8.03	43033	2.84	0.01						0.03
leaves	0	0	0.00	0.01	0.0001	0.01	0.00	0.00	0.00	0.00
roots	0	0	0.00	0.01	0.0000	0.01	#DIV/0!	#DIV/0!	0.00	0.00
#2										
Top soil	8	9496	0.62	0.01	0.0289	0.13	0.22	0.25	0.03	0.01
bottom soil	7.81	2816	0.18	0.01	0.0310	0.13	0.06	0.06	0.03	0.00
beads	8	21496	1.41	0.01						0.01
leaves	8.13	346	0.02	0.01	0.0001	0.00	2.31	2.32	0.00	0.00
roots	0	0	0.00	0.01	0.0000	0.00	0.00	0.00	0.00	0.00
Control										
Top soil	7.87	823405	53.27	0.01	0.0144	0.05	36.99	39.02	0.01	0.56
bottom soil	7.95	111386	7.28	0.01	0.0270	0.09	2.70	2.96	0.03	0.08
beads	7.84	23648	1.52	0.01					0.00	0.02
Sterilized										
Top soil	7.95	663743	43.37	0.01	0.0175	0.17	24.79	29.77	0.02	0.52
bottom soil	7.97	400113	26.21	0.01	0.0339	0.18	7.73	9.39	0.03	0.32
beads	7.89	53896	3.50	0.01						0.03

Table 41: Mass balance of GTN in surface contamination experiments

Beaker	M_{in}	Conc.	Mass degraded	% mass degraded
#	mg	mg	mg	
Yellow nutsedge				
# 1-leachate		0.31		
Top soil		0.10		
bottom soil		0.04		
beads		0.00		
leaves		0.00		
roots	7	0.00	**6.55**	**94**
# 2-leachate		0.08		
Top soil		0.15		
bottom soil		0.08		
beads		0.00		
leaves		0.00		
roots	7	0.00	**6.69**	**96**
Indian grass # 1				
Top soil		0.11		
bottom soil		0.01		
beads		0.03		
leaves		0.00		
roots	14.5	0.00	**14.35**	**99**
# 2				
Top soil		0.01		
bottom soil		0.00		
beads		0.01		
leaves		0.00		
roots	14.5	0.00	**14.48**	**100**
Control				
Top soil		0.56		
bottom soil		0.08		
beads	9	0.02	**8.34**	**93**
Sterilized				
Top soil		0.52		
bottom soil		0.32		
beads	9	0.03	**8.13**	**90**